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ASSESSING THE TOXICITY OF FRESHWATER SEDIMENTS

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Abstract - The science of sediment toxicology essentially began in the late 1970s. It was largely a product of dredging concerns and recognition of widespread contamination of sediments. During the past few years, sediment toxicity research activity has increased dramatically. Currently, most tests are of an acute nature with fewer available for determining sublethal endpoints of chronic toxicity. Test systems of single and multiple species have included most levels of biological organization in aquatic ecosystems and have been conducted in the laboratory on whole sediments, interstitial waters, elutriates, or other extractable fractions under a wide variety of conditions. Evaluations of methodological effects and comparisons with in situ toxicity using surrogate test species and indigenous communities have, on occasion, shown significant differences in test responses. These differences may be attributed to laboratory-controlled parameters (e.g., light, species, life stage, exposure conditions, test phase, spiking method); sampling and laboratory-induced disruption of sediment integrity; alteration of toxicant partitioning due to manipulations and temporal effects; and failure to recognize other influencing ecosystem variables (e.g., organism niche and life cycle, sediment partitioning and gradient dynamics, physicochemical and biological process integration, biotic and abiotic disturbances, micro- and macrobiota patches, food-web interactions). Optimizing and standardizing test methods will require further studies of these variables to improve interlaboratory comparisons and ecosystem validity. Despite the many unknowns that exist, a variety of sediment toxicity tests have been effectively used in assessing toxicant contamination by measuring the bioavailable fraction of the in-place pollutants. The optimal assays vary with the study and its objectives. Intergrative studies using several chemical, community, and toxicity measures are currently the most effective at defining ecosystem perturbations.

Keywords – Sediments Sediment toxicity Freshwater Acute Chronic Assessments

This review will focus on the relatively short history of freshwater sediment toxicity testing from a multitrophic-level perspective and also attempt to define the critical variables both in the environment and in the sampling and test design that have been shown, or thought, to influence the assessment process. As in all studies of aquatic ecosystems, the integrating factors are many and complex, crossing multiple compartments, whether or not they are recognized by the investigator. The test response of an aquatic species, community, or microcosm is meaningless if the response initiator is an unrecognized artifact of experimental manipulation. Many of the issues reviewed are also relevant to sediment-related research dealing with behavior [1], bioaccumulation [2], and genotoxicity (biomarker) studies [3] and in sediment studies of marine systems [4-6]. In marine systems there are, however, some important differences that may affect sediment toxicity and its assessment, including ionic strength-composition, salinity gradients,

lower organic carbon, species selection and test requirements, and hydrodynamics. The review has been divided into nine subject areas: history, sediment environment, ecosystem integration, system variance, sediment sampling and manipulation, assay exposure conditions, toxicity assays (arranged phylogenetically), field validation, and current applications.

THE BEGINNINGS

The science of aquatic toxicology consisted of only a handful of investigators in the 1950s with previous pioneering works by Penez and Adams (1863), Powers (1917), Belding (1927), Carpenter (1930), Ellis (1937), and Jones (1938) [7]. The scientific community remained small, as did regulatory interest, until the early 1970s, when concerns in the United States over pollution resulted in the passage of the Clean Water Act (CWA) (PL 92-500) and the formation of the U.S. Environmental Protection Agency (EPA). Before that, the problem

of elevated levels of contaminants in dredged sediments was recognized and caused the Federal Water Quality Administration to develop a few bulk (whole) sediment criteria known as the Jensen criteria [8]. The Marine Protection, Research and Sanctuaries Act (PL-92-532) mandated the EPA and U.S. Army Corps of Engineers (COE) to develop methods to evaluate dredge and fill material. The COE therefore embarked on a multimillion dollar Dredged Material Research Program that resulted in numerous technical reports addressing sediment-associated contaminant availability [8]. Significant contributions to the science were made in resulting studies of partitioning of metals in sediments; development of the elutriate technique (water exchangeable fraction) to mimic dredginginduced desorption of sediment-bound constituents; and temporal and spatial effects of dredging on water quality, fish, invertebrates, and photosynthetic organisms [9]. In 1977, the COE published guidance for ecological evaluations of dredged material, which included acute toxicity testing of whole sediment, suspended sediments, and elutriate fractions using three types of resident species [10]. It became evident from bulk sediment chemical data collected in a few locations within each state during the 1970s, as part of the CWA's monitoring programs, that many sites were contaminated with extremely high levels of metals, metalloids, pesticides, and synthetic organics [11-13]. Recognition of the widespread sediment contamination problem likely led to the increase in freshwater whole sediment and elutriate testing [14-28] and development of various bulk sediment criteria to evaluate degrees of contamination [12,29-31]. Public, regulatory, and research interest, however, focused primarily on the water column and organism health-water quality relationships until the 1980s.

Unfortunately, several of the initial sediment toxicity studies conducted during the 1970s and 1980s were reported only in the "gray" literature [10,14,16,17,27] (that is, technical reports) and thereby were not widely read by the scientific community. Early assays typically used adult life stages and consisted of short-term (acute) exposures with sample collection, manipulation, exposure conditions, or sample characteristics being relatively undefined [10,14–16,18,19,23,24,27,28]. Correlations between organism mortality and bulk sediment contaminant levels were noted in some studies with some sensitive species such as *Daphnia magna* and the burrowing mayfly, *Hexagenia limbata*, but not in others [16–24,27,28,32].

During the late 1970s and early 1980s it became apparent that the physical, chemical, and biological relationships between the sediment environment and associated contaminants were complex and variable and not easily defined or managed via selective extractions, elutriate toxicity testing, or bulk sediment criteria [8,33-35]. This widespread recognition [12] led to heightened regulatory and research activity into better ways to assess and manage sediment contamination. In the 1970s, a large amount of research was focused on sediment contaminant transport and nutrient dynamics [36-38]. In 1981, sediment quality indicators were proposed by the EPA Region VI office (F.E. Phillips, Deputy Regional Administrator; Region VI Sediment Quality Indicators Memorandum of August 19. 1981, to J. Hernandez, Deputy Administrator, U.S. Environmental Protection Agency; Dallas, TX) whereby interstitial water contaminant levels were compared to the EPA's water quality criteria. This approach suggested that interstitial water was a primary route of uptake by aquatic organisms and utilized the extensive toxicological data base incorporated into the water quality criteria. Though the data base is comprised primarily of water column organisms, the comparison was suggested as valid on the basis of water-sediment interactions. This relationship is still a point of debate. The proposed approach, however, was rejected by Assistant Administrator John Hernandez (J.W. Hernandez, Jr., Deputy Administrator; Region VI Sediment Quality Indicators Memorandum of October 16, 1981, to F.E. Phillips, Deputy Regional Administrator, U.S. Environmental Protection Agency; Washington, DC). Shortly thereafter, the EPA Criteria and Standards Division began a sediment criteria development program that focused on equilibrium partitioning of contaminants in the interstitial water. This approach also utilized the water quality criteria as a comparison, but sediment concentrations were normalized with sediment/ water partitioning coefficients [39]. Nonpolar organic (e.g., Kepone) bioavailability in sediments was shown to be driven by total organic carbon (TOC) concentrations [40], and more recently it has been suggested that metal bioavailability may be controlled by acid volatile sulfide (AVS) relationships [41].

The increased sediment research activity in the 1980s followed the work of Gannon and Beeton [14,15], who tested preference-avoidance of *Pontoporeia* sp., *Gammarus* sp., and *Chironomus* sp. larvae with contaminated sediments and found no

relationship. Magnuson et al. [16], Prater and Anderson [18,19], Birge et al. [17], and Wentsel et al. [20-22] conducted whole sediment assays in the late 1970s. In 1977, Prater and Anderson published a modified method of Fremling [42] to determine if freshwater dredged sediments were toxic [18,19]. Their system consisted of a recirculating aquarium in which dredged sediments were placed and overlain with site water. Organisms (D. magna, Asellus communis [isopod], Pimephales promelas, and H. limbata) were then exposed in the aquarium for 2 to 4 d. Poor replication and correlation with contamination might have resulted from sediment freezing, lack of feeding, and failure to use early life stages. Wentsel et al. [20-22] showed that the growth and emergence of Chironomus tentans responded to metal-contaminated sediments. Birge et al. [17] exposed amphibians and fish embryos to metal-contaminated sediments for 4 to 10 d posthatching and showed embryonic mortality and teratogenesis paralleled contaminant levels.

The increase in research is shown in Figure 1, where a clear trend of heightened activity is evidenced by publications in this journal, *Environmental Toxicology and Chemistry*. Only recently have the numbers become significant, with 51% of the 148 sediment-related and 64% of the sediment toxicity studies having been published since 1988.

Other sediment-related reviews have addressed freshwater toxicity testing [9,43–46]. The usefulness of sediment toxicity tests in the assessment and management of contaminated sediments has now been well documented [47,48]. However, a serious need exists to develop detailed standardized sediment toxicity tests [35] and better understand assay responses, their significance, and ecosystem relevance.

THE SEDIMENT ENVIRONS

The holistic (as opposed to the "reductionist") approach has been promoted recently [47,49] as the preferred method of studying aquatic ecosystems. Based on an abundance of sediment-related reductionist research on basic physical, chemical, and biological processes and considering the dynamics of stream [50-52] and lake [53,54] ecology, it is apparent that the holistic approach should be utilized when evaluating the sediment environs. Many studies have failed to recognize that a multitude of physical, chemical, and biological (microand macrobenthic) processes are integrated in the sediment as dynamic, yet structured, gradients often occurring on small spatial scales of microns to millimeters and temporal scales of minutes to months [50,51]. Given this reality it is questionable whether destruction of the sediment integrity via

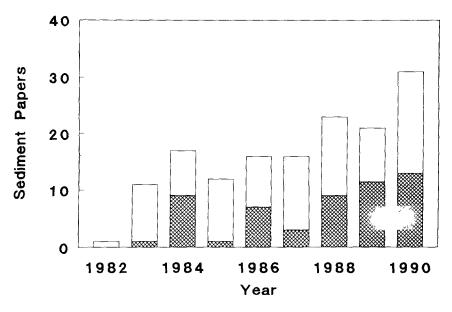


Fig. 1. Numbers of papers published in *Environmental Toxicology and Chemistry* dealing with sediments (open bar) and sediment toxicity testing (closed bar). 1982 was year one of publication (Vol. 1).

grab sampling will consistently allow laboratory studies of sediments that can be realistically extrapolated to in situ conditions.

Partitioning dynamics

Metal and metalloid dynamics between sediments and interstitial and overlying waters are particularly complex. Though many of the factors that control partitioning of organic chemicals are similar, there are important differences such as the influence of organic carbon on partitioning of nonpolar compounds [40]. For a review of organic chemical partitioning, see Di Toro et al. [55]. The movement of metals, their availability, and possible toxicity are influenced by chemical and physical reactions and factors such as oxygen/redox gradients, pH, temperature, adsorption, sedimentation, complexation, precipitation, and grain size [56]. In addition, a variety of common sediment bacterial communities can metabolize and alter metal/metalloid valence states via oxidation-reduction reactions, thereby altering chemical fate and toxicity [57,58]. Release of metals from sediments to water has been investigated primarily in dredging effect-related studies. There is little release of metals from reduced sediments in oxygenated waters during dredging operations [59]. Release is more likely after the sediments have been redeposited [59]. However, clearly dominating mechanisms do not appear to control all freshwater systems. Salomons et al. [60] observed that Cd is released from anoxic marine sediments into oxic water, but metals were also sorbed from oxic waters to freshwater sediments. Metal concentrations in water have been shown to decrease by four orders of magnitude within 1 h of dredging [57,59,61,62]. Any metals released from anoxic dredged sediments tend to adsorb onto freshly precipitated Fe/Mn oxyhydroxides [8,56,59,61,63-65]. This oxidation and release of sulfide-bound Cd and subsequent readsorption to oxyhydroxides occur in less than an hour [66,67]. Jenne and Zachara [68] state that a significant portion of dissolved metals is adsorbed irreversibly to solids within several hours. This suggests that studies on the bioavailable reversible fraction should be conducted after this initial period. However, with hydrophobic organics, equilibrium between the water and sediment phases may take days to years to reach equilibrium [69,70].

The rates of exchange (e.g., desorption) for many metals (Fe³⁺, Zn²⁺, Ni²⁺, Cu²⁺, As³⁺, Cd²⁺) are slow [57,59,61,62]. The maximum remobilization rate of Cd after dredging occurred in two to

four weeks, with maximum release after one month [66]. Remobilization (desorption) is stimulated by bacterial decomposition of substrates/ligands and formation of soluble organic compounds [56,66]. Peaks in water column metal concentration have been correlated with organic matter decomposition during the year, low flow conditions, and initial storm-water flushing [56]. Higher levels of Cd have been observed in oxic sediments overlying anoxic sediments, possibly due to sulfide removal [71]. This supports the AVS partitioning results with Cd [41].

Metals are partitioned in sediments in many forms as soluble free ions, soluble organic (low-molecular-weight humic) and inorganic complexes, easily exchangeable ions, precipitates of metal hydroxides, precipitated with colloidal ferric and manganic oxyhydroxides, insoluble organic complexes, insoluble sulfides, and residual forms [59]. The residual fraction serves as the matrix vehicle [72] and is associated with labile components (e.g., carbonates, amorphous aluminosilicates, organic matter) [73], which are coated with iron/manganese oxides and organic matter. This variable coating serves as an active sorption site for metals [56].

Free metal (e.g., Cu²⁺) is generally thought to possess the greatest toxicity [74–81], so it is important to understand binding dynamics' (such as rates) controlling conditions (such as pH, Eh) and sorption/desorption properties. In some sediments, sorption of metals is driven by amorphic oxides of Fe, Mn, and reactive particulate organic carbon [8,68]. Amorphic oxides of Si and Al as well as clay and zeolite minerals are particularly important sorbants for anionic metals and metalloids [8,68]. Use of TOC to determine sorption potential for metals is inappropriate because the aluminosilicate or carbonate coatings may isolate portions of the particulate organic carbon from the aqueous phase, thus making it "nonreactive" [68]. Manganese oxides are highly reactive, strongly sorb many ions, are involved in many redox reactions, and are common in sediments, thereby influencing mobility and fate of many pollutants [82]. Mn⁴⁺ and Fe³⁺ adsorb As3+ and are primary electron acceptors in its oxidation to As⁵⁺, a less toxic form. Mn⁴⁺ can oxidize As³⁺ and As⁵⁺ within 48 h [62]. Higher soluble As concentrations in soils are related to soluble Fe2+ [83]. Arsenic and Se are readily biomethylated and demethylated [58,84] to more or less toxic/available forms. Good correlations have been observed in bivalve tissue between As/Fe and Pb/Fe ratios and readily extractable Fe from sediments [85,86].

Another factor controlling partitioning is pH. Adsorption of Cd is easily affected between pH 7 and 9 [61], which affects formation of CdCO₃ precipitates [81]. pH and Eh changes can alter iron solubility by three orders of magnitude but rarely change the valence states [59]. Fe and Mn can desorb faster than Cu and Zn, which are insensitive to pH and oxygen changes [67].

Predicting partitioning of metals (and thus bioavailability) is difficult [56,68,69,87] due to the myriad of possible undefined processes that may simultaneously reduce and increase availability. For example, reduced sediments have shown release of Pb, Cu, and Fe while Zn and Hg decreased in overlying waters [59,64,88], and oxidized sediments released Cd, Cu, Pb, and Zn while Fe decreased [59,89]. The concentration of metal observed in interstitial waters is dependent, to a large degree, on sorption/precipitation processes. The process depends on the metal and the environment. Adsorption is complicated, being related to the type of solid (e.g., detrital matter, sand, clay), metal and solids concentration, metal speciation, and surface property changes resulting from interactions such as coagulation. In addition, there are sorption site competition and reaction kinetics of constituents in mixtures that are unknown. Calcium reduces Cd sorption by amorphic iron oxides, yet Zn is unaffected [90]. High dissolved organic matter concentrations enhance solubility and complexation of metals [61], but currently, organic ligand effects cannot be predicted.

Some studies indicate that the metal-solutionto-solids ratio has an important role in affecting $K_{\rm p}$ (sediment/water partition coefficient) [91,92]. Sharp increases in sorptions at low (400 mg/L) sediment concentrations may be due to disaggregation of sediment particles that increases exposed surface area [91,92]; however, others suggest this is experimental artifact [93,94]. In sulfidic environments Cu, Cd, and Zn concentrations are governed by the precipitation-dissolution process and are solids-concentration-independent [61], whereas in anoxic environments without sulfides, As and Cr concentrations are controlled by adsorption/desorption reactions and are solids-concentration-dependent. Partitioning coefficients vary one to three orders of magnitude in low-particle-concentration environments as a result of these interactive and nondeterministic effects [87].

Because of the heterogeneity of sediments, sediment sorption partition coefficients cannot be fully normalized by using one sediment characteristic [69]. For nonionic organics with low solubil-

ities, nonspecific van der Waal's interactions of the solute with the organic fraction of the sediment dominate [69]. With some compounds this relationship has shown that valid K_p s can be determined [95]. Equilibrium rates and bioavailability interactions are still a point of debate [96]. Rodgers et al. [97] question whether sediment organic carbon content explains the variability observed in sorption coefficients. Chemicals with high sorption coefficients were usually not acutely toxic below their solubility concentration [97]. Ionic organic compound partitioning is more complicated, being influenced by numerous charge measures such as cation exchange capacity, pH, and Eh [69]. Sorption of cationic pesticides was shown to occur on negatively charged clay and organic matter sites [98].

Jenne and Zachara [68] state that there are three critical areas where lack of data limits our ability to quantitatively describe mobility (thus bioavailability) of toxic elements in aquatic systems: (a) equilibrium and kinetics of sorption to solids; (b) thermodynamics of metal-dissolved organic carbon (-DOC) complexation, species formation, and solids solubility; and (c) kinetics of dissolution and precipitation reactions. Therefore, predictions of metal sorption have orders of magnitude uncertainty and major discrepancies with field data [68].

Gradients

Steep vertical gradients (over distances of millimeters to centimeters) exist in sediments for several parameters that influence the previously discussed partitioning processes, including oxygen, redox potential, sulfur and nitrogen species, hydrogen, methane, and labile dissolved organic compounds such as short chain fatty acid fermentation products [99-103]. Over a small range, which corresponds to the Eh profile, there is sequential consumption of different products via anaerobic respiration and methanogenesis [99]. As discussed later, these gradients may be altered by epibenthic and benthic organisms, allowing oxygen to penetrate more deeply into sediments. In regard to metal partitioning, oxygen and sulfide gradients are particularly important. Another microbial product that dominates interstitial water toxicity in some sediments [104] is ammonia. This is more of a factor in organically rich, anoxic sediments receiving nutrient inputs. Interstitial water ammonia diffuses upward and may benefit epipelic algae in euphotic zones [99]. These benthic algae (periphyton, aufwuchs) create vertical distributions of oxygen in the sediment that vary spatially (in millimeters) and temporally (from minutes to months) [99]. In lake sediment systems where biological productivity is low, the oxygen penetrates deeper (25 mm maximum) [99]. In stream systems oxygen gradients may go much deeper (centimeters) due to larger grain sizes and water exchange. These gradients can be dramatically affected by sampling, and in situ testing is recommended [99,105].

Deposition and resuspension

Another important consideration in sedimentcontaminant interactions and subsequent distribution in the aquatic system is grain size. Sediment contaminant data should be evaluated on the basis of grain size [56] correction, which reduces the inert fractions (e.g., hydrates, sulfides, amorphous and fine-grained organics). The most useful size fraction for contaminant assessments appears to be $<63 \mu m$ [106,107]. This size fraction will tend to predominate in deposition areas and be associated with contaminants if they are present. Sand has lower $K_{\rm p}$ s than silt fractions due to lower specific area and lower organic carbon content (in regard to hydrophobic solutes) [69]. Hydrodynamics plays a major role in the transport, deposition, and resuspension of the fine-grained sediments. Particle diameters of suspended solids vary over two orders of magnitude, and settling speeds in waters vary four orders of magnitude [108]. Predicting transport is complicated by the lack of understanding of aggregation/flocculation and its effect on particle sizes and settling speeds, floc disaggregation due to shear, processes governing entrainment and deposition, and turbulence description [108].

When resuspension events occur, predicting metal remobilization may be possible in site-specific studies; however, remobilization is dependent on particle residence time in the water column, which varies between sites, storms, and systems [56,66]. In most systems, however, remobilization of metals from resuspended sediments is likely to be insignificant due to the slow reaction rates [66]. Chlordane availability to *D. magna* was reduced by suspended montmorillonite clay (0% TOC), but suspended solids characteristics did not control bioavailability when their concentrations were greater than 200 to 300 mg/L [109].

Although resuspension effects appear limited if one considers the scavenging effects of solids, laboratory studies of bioturbation effects on contaminant movement and toxicity to planktonic species have shown otherwise. Bioturbation by benthic and epibenthic invertebrates occurs in many ways: by pumping pore water constituents out of the sediment into overlying waters, injecting water into

the sediment, pumping particulates to the sedimentwater interface, depositing fecal pellets on the sediment surface, and disruption of horizontal and vertical layering [110]. A bioturbation study using tubificid oligochaetes, chironomid larvae, and unionid bivalves noted substantial and different effects on sediment diagenesis and solutes exchange between sediment and water [111]. Tubificids were found in the upper 20 cm of sediment but fed primarily in the top 2 to 9 cm [112-115], which was a reducing environment. Karickhoff and Morris [113] reported that 90% of an organic chemical spiked into the biotic zone of sediment was transported by tubificids to the sediment surface in 30 to 50 d, water concentrations were increased four to six times, and fecal pellets released less than 20% of the incorporated organic.

Inorganic, physical, and microbial process dynamics are affected by bioturbation [111]. Reactive phosphate concentrations were reduced near the sediment-water and burrow water-water interfaces by the infaunal species due to sorption to ferric oxyhydroxides. Bioturbation increases aeration and thus can affect Eh and pH gradients [115-119]. The tubificids increased ammonium concentrations in the sediment and flux across the sediment-water interface and also enhanced nitrate consumption. However, the chironomids, which resided in the upper 8 to 10 cm, reduced ammonium in the top 5 to 10 cm, but it increased rapidly below the burrowed zone [111]. Findings have suggested that ammonification and nitrification are significantly enhanced in irrigated sediments [111,120,121]. The tubes were covered by diatoms, which elevated chlorophyll concentrations. The larvae thus influenced algal community dynamics by altering nutrient levels [51]. When the larvae emerged, the nutrient dynamics changed [51]. The chironomid and clam burrows were oxidized from overlying oxygenated water penetrating 6 to 8 cm. Oxygen diffusion laterally through burrow walls was evidenced by changing sediment color. The burrowing of H. limbata had significant effects on organic sorption partitioning in sediments [122]. Higher organic carbon and bacterial activity were associated within the burrow walls of H. limbata. Organic matter concentrations were doubled in lugworm burrow walls, and bacterial activity peaked in this area [123]. Tubificids, chironomids, and unionids increased sediment transport of electron acceptors (e.g., O₂, NO₃, SO₄). The increased solute flux from pore waters was greater than that predicted by pore water profiles. Some of this flux was due to altered physical structure, irrigation, radial diffusion, metabolic by-products, enhanced decomposition or mineral dissolution rates. The degree and nature of these effects were strongly influenced by the life modes of the organisms [111]. The effect of bioturbation on the overlying water environments was also evidenced by increased toxicity to water column species when burrowing organisms were present [124].

ECOSYSTEM INTEGRATION

Sediments play a major role in ecosystem processes and ecosystem health [47,53] Generally speaking, the surficial layer (upper few centimeters) is the active portion of the ecosystem, while deeper sediments are passive and more permanently in place. These deeper layers are of interest as a historical record of ecosystem activity but also may be reintroduced into the active portion of the ecosystem via dredging activities and severe storm or hydrogeological events There is a continual flux of morganic [125-127] and organic compounds [128,129] through the sediment-water interface These processes may be accelerated by biological activity (planktonic and benthic), and thus are seasonally linked [129-133], and also by other physical disturbances (e.g., flow-induced resuspension, dredging) whose temporal relationships are more chaotic in nature [134,135] Sediment and porewater phosphorus transport to overlying waters is only partially regulated by pH and Fe²⁺ and is a complex process [126,133] In some sediments, ammonia, phosphorus, and methane flux are correlated and driven by organic matter mineralization [133] Though the surficial sediment layer ecosystem is more active, it is orders of magnitude more permanent (or less active) than the overlying waters and, therefore, often serves as a better record of recent watershed activities (disturbances) than the water column This realization has led to increased sediment monitoring of contaminant concentra tions and benthic macroinvertebrate communities in recent years by federal and state regulatory agencies [12,136] The usefulness of a sedimentmonitoring station as an indicator of contaminant presence is a function of the interactions between the change in contaminant net deposition rate, sediment accumulation rate, mixing zone depth and dynamics, sampling method and frequency, type of laboratory method, and its precision and accuracy

The contamination of higher trophic levels such as fish and fish-eating birds and mammals has been linked to contaminated sediments on several occasions [43,138] In the Great Lakes, many in-

dustrial harbors have severely contaminated sediments [9,12,19,24,44,139–142], and uptake of toxicants by a primary benthic invertebrate population, *Diporeia* sp, has been well documented [143] Consequently the food chain becomes contaminated and the ecosystem subjected to an additional disturbance [52]

It has become evident that it is no longer adequate to study only separate components of the ecosystem, such as planktonic species in water-only systems or chemical dynamics in a water-only or sediment slurry system This reductionist approach [47,49] is essential for defining processes but does not provide an accurate picture of the componentecosystem interactions and, in fact, may produce misleading results Examples of this disparity are becoming increasingly obvious, particularly in the field of aquatic toxicology as more holistic types of studies are published [47,49,144] Whenever sediments are placed in toxicity assays using water column organisms and dosed with a contaminant, the nominal toxicant effect concentration is different from that of water-only exposures [81,145], if benthic invertebrates are added to the same system, then water column species may be adversely affected [124]

Numerous studies [144-157] have demonstrated the importance of using several species to evaluate ecosystem contamination because species sensitivity varies between toxicants and, in environmental settings, is likely unpredictable without previous study Many test species/communities and associated endpoints have been recognized as useful surrogates in water, effluent, and sediment toxicity studies D magna, Ceriodaphnia dubia, P promelas, Tubifex tubifex, C tentans, Chironomus riparius, H limbata, Hyalella azteca, Panagrellus redivivus, Selenastrum capricornutum, Lemna minor and Microtox®, and indigenous bacterial, protozoan, macrobenthic invertebrate, or planktonic communities A review of studies that used multiple assays (e g, [17-19,24-28,74,105,139,140,144, 146,153–178]) revealed that *each* test species and/or endpoint was reported as the most sensitive or of equal sensitivity at one time or another Other comparisons have indicated no one species to be most sensitive to all chemicals [179] This supports the premise that a multitrophic-level test battery is essential in assessments of aquatic ecosystems and sediment quality

The majority of these studies dealing with aquatic toxicity to a surrogate species (or a small number of species) have not attempted to investigate ecosystem interactions a priori, such as ecosys-

tem energetics or stress-productivity-predation relationships. Rather, surrogate responses have simply been quantified on the basis of sample toxicity and effects extrapolated to in situ conditions. Although these exercises may satisfy the study objectives of defining sample toxicity to the test species, they do little to document or define ecosystem disturbance. This is not a reflection on the quality of the science published to date but an indication that the science is as yet in its infancy.

Cairns [180] has called for ecotoxicologists to recognize ecology more in their studies of environmental toxicity. Ecological processes can be ignored, to a degree, whenever acute toxicity scenarios are studied, such as sediments that are severely degraded. However, "significant cases of acute toxic effects have been encountered infrequently" [181], and the more common situations in which effects and zones of contamination are gray [47,181] dictate that natural and anthropogenic effects be separated. This cannot be done accurately without an understanding of ecosystem dynamics such as spatial and temporal variance of chemical, physical, and biological systems and their interactive processes.

Community ecology in lotic and lentic systems has progressed substantially in recent years [50-52,182]. "Biotic dynamics and interactions are intimately and inextricably linked to variation in abiotic factors" [183], and lotic systems are not in equilibrium due to natural disturbances that may occur frequently or infrequently [52]. Disturbance can be defined as a discrete event that alters community structure and changes the physical environment and resource availability. These disturbances vary in type, frequency, and severity, both among and within ecoregions. The frequency and intensity of disturbances cannot be predicted [52]. Intermediate levels of disturbance maximize species richness [52]. This relationship to diversity has been described in a dynamic equilibrium model [184] whereby the frequency of disturbance controls whether competitive species and long-life-cycle species exist. Equilibrium or steady-state conditions will tend to occur if disturbances are infrequent, thus excluding opportunistic species [185]. In stream ecology, disturbance is the dominating organizing factor, having a "major impact on productivity, nutrient cycling and spiraling, and decomposition" [52]. Disturbances such as storm events or the presence of toxicants can eliminate biota [183]. Recovery and succession of these systems between disturbances are typified by recurrent or divergent patterns [51,52]. Despite this inherent

variability, benthic communities have been used effectively to classify community structure and functioning in aquatic ecosystems [182].

In some systems "bottom-up" effects have been observed where algal succession or community composition alterations affect zooplankton grazers [186-189]. Interruption of microbial cycling processes reduces ecosystem productivity, alters sediment redox, produces anoxia, and increases H2S production and acidity [188,189]. When benthic invertebrate and protozoan cropping of bacteria is discontinued due to contaminant-induced lethality, the sediments serve as a carbon sink [188,189], so organic carbon and nutrients necessary for secondary productivity are unavailable and food-web alterations are likely [188,189]. The sediment bacterial community is available to sediment ingesters, and bacterial production is transferred relatively efficiently to fish through a single intermediate consumer such as Chironomus [190]. In streams, bacterial productivity may be greater from sediments than total primary production [191]. Benthic shredders (amphipods) could differentiate between fungal species colonizing detritus. So a change in the fungal community could alter organic matter processing rates [192].

If one is concerned with energy flow then the bottom-up approach dominates; however, predator removal (top-down) will alter community structure, productivity, or biomass [187]. This reemphasizes the complex and simultaneous functioning of ecosystem relationships.

Maximum productivity occurs at all trophic levels, and maximum energy flow through the food web occurs when predation intensities are intermediate throughout the system [54]. Nutrient excretion by zooplankton is a major recycling process [186] that is affected by fish predation [187]. This suggests consumers regulate primary productivity top-down in some systems [54].

SYSTEM VARIANCE

The major effect of natural and anthropogenic disturbances on aquatic ecosystems is the increase of spatial and temporal variance under equilibrium conditions to much higher levels. Spatial and temporal dimensions span 16 orders of magnitude in stream ecology [50,51]. Some suggest that spatial heterogeneity enhances the ability of an ecosystem to resist and recover after a disturbance [193]. Significant spatial variance in sediments is common [194,195]. Each level of the system has different dimensions and variances associated with it and interacts simultaneously with other ecosystem levels

and their respective dimensions and variances. This complex reality is difficult, if not impossible, to define accurately but must be considered in all assessments of sediment quality or ecosystem health.

Orians [196] stated that one of the greatest challenges in ecology (and ecotoxicology) is bridging the conceptual gap between micro- and macroecology. Aquatic systems can be considered as a mosaic of "patches" [51]. "A patch is a spatial unit that is determined by the organism and problems in question" [51]. The heterogeneous environment has highly clumped distributions (patches) of organisms whose spatial and temporal patterns and relationships change seasonally due to factors such as food (resource) patterns [197]; therefore, such patches pose severe sampling problems. The appropriate sampling scale will depend on the organism's size, density, distribution, and life cycle, as well as the question being asked [51], which, unfortunately, is often not considered. Aquatic ecosystems are open nonequilibrium systems [51,54] in which patches are in transitory steady state with other patches [198]. Different life histories and variable interactions between species may prevent equilibrium [54].

The influence of storm events and watershed characteristics on chemical element dynamics is poorly understood, particularly because some are lumped into operationally defined units such as DOC or TOC [199]. Significant heterogeneity (62–100%) has been observed between adjacent sediment cores in concentrations of organic matter, water, and total phosphorus [200]. Some heterogeneity is likely due to invertebrates [201], sediment transport by currents, and small-scale variations in bottom profiles [194,200].

Varying tissue residue levels of Cd in *H. azteca* [202] and of Hg in plankton and benthos [130] were related to hardness and both seasonal and regional factors, respectively. Chironomid uptake of Hg was limited by colloidal hydrated oxides of iron (FeOOH), and MnOOH affected oligochaete, nematode, and pelecypod uptake [130].

Di Toro et al. [41,55] found AVS concentrations in sediments also varied seasonally, peaking in late summer to early fall and again in spring, which appears to correlate with productivity inputs. This would be expected according to microbial activity studies in which peak activity in sediments correlated with seasonal plankton blooms/organic carbon inputs [129,132]. Microbial processing contributed substantially to carbon cycling in the summer, but 95% of the primary production went to the benthos in winter [131].

Sediment microbial activity, as previously discussed, varies significantly with sediment depth due to gradients of natural electron acceptors [99–103]. This vertical variance has also been linked to toxicant deposition patterns [195]. A contaminated stream station showing homogeneous particle size distributions of sand and silt and water content was sampled on both a 0.04-m² and a 1-m² grid with a hand corer and Ekman dredge. Indigenous sediment microbial activity (β -galactosidase, β -glucosidase, alkaline phosphatase, and dehydrogenases) and mortality of D. magna and C. dubia were not significantly different in subsamples; however, spatial differences of 100% or greater were observed on small and large scales both horizontally (20 cm-10 m) and vertically (4 cm intervals to 20 cm depth) [195].

In stream benthic communities, hydraulics appear to be more important than substrate in determining distribution [203]. Small-scale sampling is more apt to define meiofaunal patches than large-scale sampling, which homogenizes patchiness and [197] thus ameliorates significant differences. The replicate number needed to obtain a given precision decreases with increased density and sampler size, and the optimal sampler size (considering cost and precision) depends on mean density [204].

SEDIMENT SAMPLING AND MANIPULATION

Given the previous discussion, sampling and testing of sediments are difficult if the investigator is attempting to define in situ conditions. It is impossible to sample sediment without some degree of disruption, as the very process of removing sediment is disruptive. Few studies have focused on sampling and sample manipulation (e.g., spiking) effects on resulting toxicity responses [205,206]. A standard guide was recently published by the American Society for Testing and Materials (ASTM) concerning collection, storage, characterization, and manipulation of sediments for toxicological testing [207]. Other regulatory guidance documents exist that are concerned, in part, with sediment collection and characterization procedures [207]. The ASTM guide attempts to standardize sediment collection and handling methods by presenting limited options with their associated strengths and weaknesses. However, it is apparent that the field of sediment assessments is in its infancy, and the guidance information is of a provisional nature.

Sampling

Choosing the most appropriate sediment sampler for a study will depend on the sediment's characteristics, the required efficiency, and the study objectives. Several references are available that discuss the various collection devices [194,207–212]. The efficiencies of these samplers for benthic collections have been compared, and, in general, the grab samplers are less efficient collectors than the corers [207].

The principal disadvantage of dredge samplers varies; common problems are shallow depth of penetration and presence of a shock wave that results in loss of the fine surface sediments. Dredge samplers that quantitatively sample surface sediments have been described [212]. The depth profile of the sample may be lost in the removal of the sample from the sampler. Dredge sampling promotes loss of not only fine sediments, but also water-soluble compounds and volatile organic compounds present in the sediment [207].

Studies of macroinvertebrate sampling efficiency with various grab samplers have provided useful information for sampling in sediment toxicity and sediment quality evaluations. The Ekman dredge is the most commonly used sampler for benthic investigations [208]. The Ekman's efficiency is limited to less compacted, fine-grained sediments, as are the corer samplers. The most commonly used corer is the Kajak-Brinkhurst, or hand, corer. In more resistant sediments the Petersen, Ponar, and Smith-McIntyre dredges are used most often [208]. Based on studies of benthic macroinvertebrate populations, the sediment corers are the most accurate samplers, followed by the Ekman dredge, in most cases [208]. For resistant sediments, the Ponar dredge is the most accurate and the Petersen the least [208]. A comparison of sampler precision showed the van Veen sampler to be the least precise; the most precise were the corers and Ekman dredge [208].

Corer samplers also have limitations [207] in some situations. Most corers do not work well in sandy sediments; dredge samplers or diver-collected material remain the only current alternatives. In general, corers collect less sediment than dredge samplers, which may provide inadequate quantities for some studies. Small corers tend to increase bow waves (disturbance of surface sediments) and compaction, thus altering the vertical profile. However, these corers provide better confidence limits and spatial information when multiple cores are obtained [197,208,213-215]. Care must be taken in subsampling from core samples, because surface sediments might be disrupted in even hand-held core collection [216]. Rutletdge and Fleeger [216] recommend subsampling in situ or homogenizing

core sections before subsampling. For some studies it has been advantageous or necessary to composite or mix single sediment samples. Composites usually consisted of three to five grab samples [207].

Samples are frequently of a mixed depth, but a 2-cm sample [35] is recommended and is the most common depth obtained, although depths up to 12 m have been used in some dredging studies. The upper 2 cm represents the most biologically and hydrodynamically active portion of the sediment and is the appropriate sample depth for many assessments. However, for studies concerned with historical pollution, depositional patterns, or dredging issues, it is necessary to sample deeper sediments.

Reference and control sediment

Assessment of in situ sediment toxicity is aided by collection and testing of reference and control samples [207]. A reference sediment may be defined as a sediment possessing similar characteristics to those of the test sediment but without anthropogenic contaminants. However, it is not unusual for nearby reference sites to have some degree of contamination. Sediment characteristics such as particle size distribution and percent organic carbon should bracket these of the test sediment [207]. In some situations, the reference sediment might be toxic due to naturally occurring chemical, physical, or biological properties. For this reason, it is important also to test the toxicity of control sediments. A control sediment might consist of natural or artificially prepared sediments of known composition and of consistent quality that have been used in prior sediment toxicity tests or culturing and for which baseline data exist that show they do not cause toxicity [207]. Control sediments have been successfully used in toxicity evaluations [40].

Storage

Drying, freezing, and cold storage conditions affect bioavailability [207]. Often the storage time of sediments used in toxicity tests was not specified and where specified ranged from a few days [205] to one year [217]. Storage of sediments after arrival at the laboratory was generally by refrigeration at 4°C [9,44,145,146,174,184,197,198,201,207, 217]. Significant changes in metal toxicity to cladocerans and microbial activity have been observed in stored sediments [156,206]. Recommended limits for storage of metal-spiked sediments have ranged

from within 2 [206] to 5 [218] to 7 d [35] to less than two weeks [207,219]. A study of sediments contaminated with nonpolar organics found that interstitial water storage time did not affect toxicity to polychaetes whenever samples were frozen [220]. However, organic contaminant availability has been shown to change with storage [143].

Sediment handling and storage may alter the AVS concentrations that potentially affect metal availability. Cadmium toxicity in sediments has been shown to be related to AVS complexation [55,221]. Whenever anoxic sediments are exposed to air during collection and processing, AVSs are volatilized. Acid volatile sulfides are a reactive solid-phase sulfide pool that apparently binds some metals, thus reducing toxicity [55,221]. If a study objective is to investigate metal toxicity and the sediment environment is anoxic, then exposure to air might reduce or increase toxicity due to oxidation and precipitation of the metal species or loss of AVS complexation. It is generally agreed that sediments to be used for toxicity testing should not be frozen [222] and kept at 4°C under zero air space or N₂ for less than two weeks before testing [35].

Although risking changes in sediment composition, several studies elected to freeze samples [18,24,220,223]. Fast-freezing of sediment cores has been recommended for chemical analyses; however, this alters sediment structure and profile distortion occurs [216]. Freezing and thawing appeared to increase release of soluble organic carbon [220]. Freezing in anoxic atmospheres has been reported to inhibit oxidation of reduced Fe and Mn compounds [68]. It has also been recommended for stored sediments that are to be analyzed for organics and nutrients [224].

Interstitial water chemistry changed significantly after 24 h storage [225], even when stored at in situ temperatures [225,226]. Coagulation and precipitation of the humic material were noted when interstitial water was stored at 4°C for more than one week [227]. Oxidation of reduced arsenic species in pore water of stored sediments was unaffected for up to six weeks when samples were acidified and kept near 0°C without deoxygenation. When samples were not acidified, deoxygenation was necessary [228].

Interstitial water collection

Isolation of sediment interstitial water has been accomplished by several methods: centrifugation, squeezing, suction, and equilibrium dialysis [207].

In general, methods for recovery of relatively large volumes of interstitial water from sediments are limited to either centrifugation [229] or squeezing [230]. Some pore water constituents, for example, DOC or dimethylsulfide, might be significantly affected by the collection method [8,231]. Other constituents such as salinity, dissolved inorganic carbon, ammonia, sulfide, and sulfate might not be affected by collection methods, providing oxidation is prevented [231]. If sediments are anoxic, all steps involved in sample processing might need to be conducted in inert atmospheres to prevent oxidation of reduced species [231–233].

If interstitial water is collected by centrifugation and filtration, then effects on the interstitial chemistry (and thus toxicity) need to be considered after centrifugation [205]. Centrifugation followed by 2-µm filtration yielded similar metal concentrations to those of dialysis methods [234]. However, filtration with glass fiber or plastic filters is not appropriate in some cases and has been shown to remove contaminants [235]. Centrifugation at 7,600 g with glass contact only was shown to be superior to filtration methods [235]. Others have produced contrary results, recommending filtration with polycarbonate filters [236]. Filtration is normally conducted to remove particles with a 0.45- μ m pore size, however 0.20- μ m or smaller pore size membranes have been recommended [68]. Centrifugation may not remove dispersible clays, which, because trace metals concentrate on solids, may have significant effects on sorption studies [68].

Elutriates

Many studies of sediment toxicity have been conducted on the elutriate or water-extractable phase [10,44,237]. This method was developed to assess the effects of dredging operations on water quality. Sediments are typically shaken in site or reconstituted water (1:4 v/v ratio) for 30 min. The water phase is then separated from the sediment by centrifugation, followed by filtration of the supernatant through a 0.45- μ m filter when conducting some tests such as algal growth assays. Filtration may significantly reduce biological effects [238] by removing particulate-associated contaminants and dissolved contaminants that bind to the filter matrix. The method of elutriation affects metal speciation, ammonia concentration, pH [239], and toxicity [238]. The solids-to-solution ratio affects sorption rates [65,69] and should be considered when doing sediment extractions such as preparation of elutriates, but may not be a critical factor in whole sediment tests [195] where there is no mixing.

Spiking

A large number of marine and freshwater sediment toxicity studies have used spiked (dosed) sediments so that pure chemical fate or effects can be examined. It has been proposed as a method to aid in development of sediment criteria [39] and is representative of recent discharges [35]. The method of spiking has varied with both organics and metals. Organic compounds are generally added via a carrier solvent such as acetone or methanol to ensure solubilization and their remaining in solution during mixing. Whereas organic compounds are generally added in an organic carrier, metals are generally in aqueous solutions. Compounds are also added to water overlying sediments, and the compound is allowed to sorb with no mixing [207]. Occasionally the carrier is added directly to sediment [207], then the carrier is evaporated before addition of water. This approach seems to result in sorption to different sites from those of dosing under aqueous conditions. In most cases, either the compound is coated on the walls of the flask and an aqueous slurry (sediment and water in various proportions) added or the carrier-containing mixture is added directly to the slurry. A variety of methods have been used to spike sediments with metals. The two principal methods are (a) metal is added directly to the sediment, which is mixed, and then water is added [207,240] or (b) metal is added to the overlying waters [145,207]. Thorough mixing of spiked sediments has been accomplished with the rolling mill technique and Eberbach and gyrorotary shakers [207].

The time between the spiking of the compounds and the use of the test sediment has been variable [207] and does seem to affect the biological availability of compounds [32,143,205,241]. Equilibration and mixing conditions vary widely in spiking studies. The duration of contact between the chemical and sediment particles can affect both the partitioning and the bioavailability of the toxicant, as discussed in preceding sections. This effect apparently occurs due to an initial rapid labile sorption followed by movement of the toxicant into resistant sorption sites on or in the particle [70,242]. In addition, it is important to recognize that the quantity of spiked toxicant might exceed the complexation capacity of the test sediment system and not allow reactions to attain equilibrium. These phenomena will complicate test result interpretation [206,243].

Sediment dilution

Another manipulation of sediments for toxicity testing is sediment dilution. In order to obtain concentration-effect information in whole sediment toxicity evaluations, differing concentrations of the test sediment should be used [244,245]. Currently, there is little information available on the most appropriate method for diluting test sediments to obtain a graded contaminant concentration or concerning the methodological effects of such a dilution. A "clean" noncontaminated sediment may be used as the "diluent," which optimally has physiocochemical characteristics similar to those of the test sediment, such as organic matter/carbon and particle size, but does not contain elevated (above background) levels of the toxicants of concern [207,244-246]. However, adding clean sediment increases fresh sorptive sites for contaminants, thus reducing the biological effect further than simple dilution. Others have diluted test sediments with water [156,247] or clean sand [244] or have diluted pore water [245]. In all dilution methods, both the effect of contact time of the interstitial water and sediment (i.e., equilibrium) and the effect of disrupting the sediment's integrity on toxicant availability must be considered. Dry weight is often used as the standardization unit in sediment studies [239]; however, it has been shown to be inappropriate when determining bacteria, organic matter, and enzyme activity relationships both directly [248] and indirectly [249]. Biologically, relationships based on dry weight may be artifactual. and volumetric (areal) units incorporating water content and bulk density appear to be superior as a sediment standardization unit [248]. This issue should be considered when determining concentration-effect levels.

ASSAY EXPOSURE CONDITIONS

Given the sensitive and tenuous nature of sediment integrity, exposure conditions are particularly crucial in determining toxicant behavior and organism/community response. Parameters of concern include time of exposure, feeding, water:sediment contact time, the chemical/physical environment (e.g., light, temperature, dissolved oxygen), and the sediment test phase (whole, interstitial, or extractable).

Time

Most marine and freshwater sediment toxicity testing has been limited to acute testing in which exposure periods typically were 15 min for Microtox; 48 h for cladocerans; 96 h for fish; and 4 to 10 d for amphipods, oligochaetes, chironomids, and Ephemeroptera. Greater sensitivity to toxicants occurs with extended exposure [32,250-253]. However, only a limited amount of freshwater subchronic and chronic toxicity testing has been conducted and has usually consisted of algal metabolism (24 h) and growth (96 h), cladoceran reproduction (7 d), amphipod reproduction and growth (28 d), oligochaete growth (10 d), chironomid growth and emergence (10-15 d), and fish terta and growth (7 d). There is a continuing debate in aquatic toxicology over the definitions, adequacy, and/or relationships between acute, subchronic, and chronic toxicity testing [254-256]. Early life stage assays that monitor fecundity and growth are more sensitive than survival studies of adults [257]. The sensitivity of many molecular and cellular endpoints is greater than community structure and ecosystem function endpoints (Fig. 2); however, determining their ecosystem significance is difficult at this point

in time. There are obvious advantages of conducting subchronic tests (e.g., shorter testing period is less resource-intensive, allowing more testing) and chronic tests (e.g., no extrapolation from shorter test exposure periods necessary) [255,256]. In addition, different lethal or sublethal metabolic endpoints can be studied, so both types of assays are useful and necessary.

Feeding

The same testing factors (feeding, physical and chemical conditions) that have been recognized as important in controlling toxicity responses in effluent pure chemical and water column assays [258] are also important in assays of sediment toxicity. However, some different considerations and parameters do exist, such as the changing through time of water quality [259]. Reduced feeding did not reduce amphipod survival but did delay midge emergence. Increased feeding required flow-through conditions to inhibit growth of mold-bacteria on the sediment surface [259].

Cladocerans and fish do not collect food as efficiently when sediments are present in the test beaker, so growth may be greater in control or water-phase exposures [260,261]. Feeding algae or

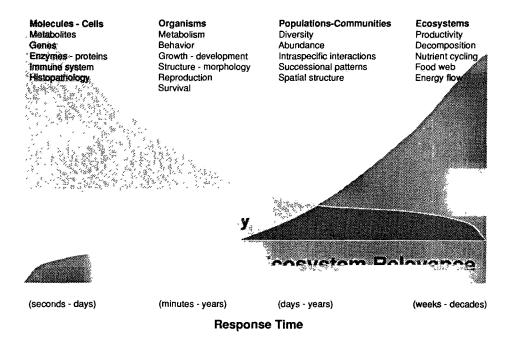


Fig. 2. General relationship between biological endpoints, level of toxicant sensitivity, ecosystem relevance, and response time (modified from [464]).

Table 1. Sediment phases used in toxicity tests

Phase	Strengths	Weaknesses	Routine Uses
Extractable phase (XP) (solutes vary)	 Use with all sediment types Sequentially extract different degrees of bioavailable fractions Greater variety of available assay endpoints Determine dose response 	• Ecosystem realism: Bioavailability unknown, chemical alternation	Rapid screen Unique endpoints, so component of test battery
Elutriate phase (EP) (water extractable)	 Use with all sediment types Readily available fraction Mimics anoxic toxic environmental process Large variety of available assay endpoints Methods relatively standardized Determine dose response 	 Ecosystem realism: Only one oxidizing condition used; only one solid:water ratio; exposure for extended period of one-phase condition that never occurs in situ or never occurs in equilibrium in situ. Extract conditions vary with investigator Filtration affects response, sometimes used 	 Rapid screen Endpoints not possible with WS Dredging evaluations
Interstitial water (IW)	 Direct route of uptake for some species Semidirect exposure phase for some species Large variety of available assay endpoints Methods of exposure relatively standardized Determine dose response Sediment quality criteria 	 Cannot collect IW from some sediments Limited volumes can be collected efficiently Optimal collection method unknown, constituents altered by all methods Exposure phase altered chemically and physically when isolated from WS Flux between overlying water and sediment unknown Relationship to and between some organisms uncertain: burrowers, epibenthic, water column species, filter feeders, selective filtering, life cycle versus pore water exposure 	Rapid screen Endpoints not possible with WS Initial surveys Sediment criteria
Whole sediment (WS)	 Use with all sediment Relative realism high Determine dose response Holistic (whole) versus reductionist toxicity approach (water, IW, EP, and XP) Sediment quality criteria may be determined Use site or reconstituted water to isolate WS toxicity 	 Some physical/chemical/microbiological alteration from field collection Dose-response methods tentative Testing more difficult with some species and some sediments Few standard methods Indigenous biota may be present in sample 	Rapid screen Chronic studies Initial surveys Sediment criteria
I n situ ^a (NS)	 Real measure integrating all key components, eliminating extraneous influences Sediment quality criteria may be determined Resuspension/suspended solids effects assessed 	 Few methods and endpoints Not as rapid as some assay systems Mesocosms variable Predation by indigenous biota 	Resuspension effects Intensive system monitoring Sediment criteria

1598

^aOrganisms exposed in situ in natural systems, pond/stream mesocosms, or lake limnocorrals.

other organic matter (e.g., yeast, Trout Chow®) can alter dissolved oxygen, pH, and/or toxicant availability, which should be monitored, standardized, and related to in situ conditions wherever possible.

Other physical parameters

Turbidity due to nonsettleable clays, dissolved organics, algae, and other suspended/resuspended solids should be monitored because it will affect toxicant availability [109] and change through time, as previously discussed. Temperature will affect fugacity via thermodynamic and metabolic rate relationships. Natural light contains UV wavelengths, which have been shown to trigger photoinduced toxicity in the presence of polycyclic aromatic hydrocarbons (PAHs) [262].

Sediment contact

Significant correlations between organism response (sample toxicity effect level) and sediment contaminant concentrations have been made with a variety of test systems (e.g., static, recirculating, static renewal), test species (e.g., planktonic, benthic invertebrate, microbial), and sample phases (Table 1). Sediment: water contact time in sediment toxicity assays may exert substantial effects on overlying water quality and, therefore, organism response [145]. Sediment oxygen demand (biochemical and chemical) can be significant in some sediments rich in nutrients and reduced substances [263], requiring aeration of overlying water [264]. Dissolution of sediment components such as carbonates may elevate hardness [253,259], which would affect the availability of some metals such as Cd, Cu, and Pb. Whole sediments spiked with arsenite, Cu, or Cd have shown decreasing toxicity and overlying water concentrations of metal decrease with extended exposure periods [145,205, 265,266]. Disturbance of redox gradients and increased oxygenation may result in reduced levels of AVS and, thus, possible release of free/available metal species [41]. Exposure conditions have consisted of static [267], recirculating [19,27,75], staticrenewal [267], and flow-through systems [27,238, 259,267], and system comparisons have shown significant differences in toxicity response [219, 238,253] with static being the worst case [219,238]. Higher survival in flow-through whole sediment assays, as compared to static exposures, has been attributed to flushing of desorbed contaminants from overlying water or more stable (optimal) water quality [238,259]. Overlying water hardness, alkalinity, and conductivity more than doubled during 29-d static exposures [259].

In whole sediment assays a 1:4 ratio of sediment:water has been common [219]; however, the Prater-Anderson recirculating system has been used at a ratio of 1:9.5 [19]. The 1:4 ratio probably originated from the COE elutriate preparation procedure. A study of Se-spiked sediment showed the sediment:water volume or surface interface area ratios did not affect *D. magna* survival until extreme ratios were used, such as 3:1 or 1:8 [206]. This finding may not be universal because partitioning dynamics are complex and chemical-dependent.

Test phase

Perhaps the most important issue in sediment toxicity testing is the appropriate test phase to use. Test phase systems can be categorized as follows: extractable (solute other than water) phase (XP), elutriate (water-extractable) phase (EP), interstitial water phase (IW), whole sediment (WS), and in situ (NS) assays. Each has associated strengths and weaknesses that prevent recommendation of any one system to meet all study objectives. The issues discussed previously regarding sediment integrity and toxicant sorption/desorption are particularly pertinent when attempting to interpret assay responses between different test phases. These considerations are summarized in Table 1.

Few studies have compared test phases as treatments [175,238]. Some studies have compared phases but used different assays [175,245], which does not allow a direct comparison of phase effects on toxicity. The elutriate phase has been shown to be more toxic [268] and less toxic [140,175,238] than other phases. In studies of four areas in the Great Lakes [140,175] and one stream in Ohio [238], the elutriate fraction was always less toxic than whole sediment assays using the same endpoints. Some sediment toxicity effects are associated with the whole phase only [74]. Interstitial waters, however, were more toxic or of equal toxicity to whole sediment [238]. The greater toxicity may be due to elevated ammonia concentrations [104] that are diluted in overlying waters in whole sediment assays. Higher metal concentrations have been observed in interstitial waters compared to elutriates [25,33]. Benthic species exposed in systems void of sediments may be stressed [46,245] and thus affect the toxicity-effect level. Knezovich et al. [269] state that an organism morphology, ecological niche, feeding mechanism, and physiology will determine toxicant, uptake, pathway, and thus, hazard. For example, oligochaetes are sediment ingesters, whereas many benthic and epibenthic species are filter feeders [40] and thus are exposed to interstitial and overlying waters to varying degrees [40,270]. It is likely that no consistent relationship between relative toxicity of all interstitial, elutriate, and whole sediment assays will ever exist due to the multitude of physicochemical and biological process variables.

Some acute toxicity assays using benthic invertebrates have been conducted in sediment-free systems such as interstitial water, elutriate phase, or spiked waters [245,252]. As these species (such as *H. azteca, Chironomus* sp., or *H. limbata*) require substrate contact and/or burrowing capabilities during their life cycles [271,272], its absence induces artificial and perhaps stressful conditions [45]. Stress has been observed in exposures greater than 48 h by decreased control survival or cannibalism. The relationship of this unnatural stress factor on acute effect-level determinations is unknown but should be considered.

Considerations in microbial assays

Microbial (bacterial) systems are particularly sensitive to test conditions [273]. Indigenous enzyme activity and luminescence are significantly affected by the sample diluent type [104,273–275]. Dehydrogenases are oxidoreductases that are active in the electron transport system and localized in cellular membranes. They are, therefore, sensitive to osmotic changes potentially introduced by the sample diluent and the extracting solution, which lyses the membranes [273]. Bioluminescence in the Microtox assay is also affected by the sediment extraction solution [274], and the toxicity of some compounds such as ammonia is quantified better using sucrose as a diluent rather than sodium chloride [104]. The type and quantity of labeled substrate (such as triphenyl tetrazolium chloride or ¹⁴C algal hydrolysate) used in determinations of sediment microbial activity are also controlling factors in assay response. Other factors that have been shown to affect enzyme activity determinations are sample storage [249,275,276] and false positives (elevated activity) due to abiotic chemical reduction of enzyme substrates [167,277]. Because indigenous microbial populations can change rapidly. both in community composition and in metabolic rate, it is necessary to conduct assays as soon as possible after sample collection and use a short exposure (incubation) time [275].

TOXICITY ASSAYS

A wide variety of toxicity assays used to evaluate water column, effluent, and pure chemical toxicity have been modified for laboratory and in situ testing of whole sediments or used, unmodified, in assays of XP, EP, or IW (Table 2). Some water column assays have used overlying water or been placed in situ, above the sediment, in evaluations of sediment quality [278,279]. Few benthic organism assays exist, but they have been widely used. Most studies have used single species exposures, but use of multispecies test systems (e.g., protozoan colonization) have advantages of more realistic interactions and ecosystem-relevant (structure-function) endpoints [4,280,281]. There are numerous assay characteristics that should be considered when selecting the optimal toxicity assay to meet assessment objectives (Table 3). No one assay is superior in any one characteristic for all study objectives. Because verification components such as ecosystem relevance, sensitivity, and discriminatory ability are so critical in the assessment process, multiple species and endpoints should be incorporated in thorough assessments of sediment contamination. The characteristics of an ideal sediment assay and indicators of ecological change have been discussed by others [282-284]. Examples of the various assays, assay conditions, and significant findings are reported here.

Bacteria

Microbes are a critical component of detrital breakdown [285]. Their role in nutrient mineralization, nitrogen fixation, sulfate reduction, and carbon cycling through the detrital food chain is well established [188,189,285]. Toxicant transformation kinetics in sediments appear to be linked to organic carbon cycling [127]. Many of the hydrolases and oxidation-reduction enzymes measured in microbial assays are involved in a variety of major metabolic systems common at all trophic levels and critical in biogeochemical cycling [188]. The productivity of fisheries has been tied to the detrital food chain [188,189].

Monitoring microbial responses has been recommended as an early warning indicator of ecosystem stress [286] and a means of establishing toxicant criteria for terrestrial and aquatic ecosystems [287]. Resulting changes at the species level should be accompanied by changes in respiration and/or decomposition rates [286]. The usefulness

Table 2. Representative freshwater and sediment toxicity tests

Biological level	Assay/organism community	Endpoint
Amphibians	Xenopus laevis	Embryo-larval survival, terata
Fish	Pimephales promelas	Embryo-larval survival, growth, terata
Zooplankton	Colpidium campylum Brachionus sp. Protozoan colonization Daphnia magna Ceriodaphnia dubia	Growth Survival Structure indices, respiration Survival, reproduction Survival, reproduction
Benthic invertebrates	Panagrellus redivivus Caenorhabditis elegans Tubifex tubifex Stylodrilus heringianus Hyalella azteca Pontoporeia hoyi (Diporeia sp.) Corbicula fluminea Anodonta imbecilis Chironomus tentans C. riparius Hexagenia limbata Macrobenthic community	Survival, growth, molting Survival Survival Survival, avoidance, reworking rate, growth Survival, growth, reproduction Survival, avoidance Survival, growth Survival Survival, growth, emergence Survival, growth Survival, molting frequency Community/population indices
Microbes	Microtox (<i>Photobacterium phosphoreum</i>) Alkaline phosphatase (sediment community) Dehydrogenase (sediment community) β -Galactosidase (sediment community) β -Glucosidase (sediment community)	Luminescence Enzyme activity Enzyme activity Enzyme activity Enzyme activity
Phytoplankton	Selenastrum capricornutum Natural phytoplankton	Growth, ¹⁴ C uptake Fluorescence, structure-species abundance
Macrophytes	Lemna minor	Growth (frond number), chlorophyll <i>a</i> , biomass
	Hydrilla verticillata	Shoot length, root length, dehydrogenase activity, chlorophyll <i>a</i> , peroxidase

of monitoring the microbial community is due, in part, to its ability to respond so quickly to environmental conditions (e.g., toxicant exposure) and the major role it plays in ecosystem biogeochemical cycling processes [188,287] and the food web [189, 1901. Indigenous microbial activity in waters and whole sediments has been shown repeatedly to be sensitive to stream degradation, as evidenced by correlations with macrofaunal community indices and toxicant concentrations [146]. These effects were often observed at low μ g/L concentrations of metals and/or synthetic organics. Measurement and/or interpretation of ecosystem function indices and their significance is often difficult, particularly when information on the indigenous communities is unavailable. When macro- and meiobenthic invertebrate and protozoan cropping of bacteria is removed, organic carbon and nutrients may become unavailable and impacts to the remainder of the food chain are likely [31,189].

When investigating chronic toxicity and other

early warning indicators of toxicant stress, stimulatory effects are often noted at low toxicant concentrations in fish, cladoceran, oligochaete, algal, macrophyte, and microbial indicator assay responses [140,146,288,289]. This phenomenon is common when using microbial and photosynthetic organisms as indicators. Stimulatory effects can be attributed to nutrients, adapted microbial communities, the Arndt-Schultz phenomenon, and/or feedback mechanism disruption [290-292] whereby low levels of toxicants increase metabolic processes. This latter possibility has been reported elsewhere in aquatic evaluations [157]. Pratt et al. [292] suggest that elevated structure and function responses are initial stress indicators that probably reflect a disruption of normal feedback mechanisms controlling nutrient dynamics and species interactions. Stimulation or inhibition of activity may also result when carbon or nutrient substrates are altered [30,31,189], so that one enzyme system (e.g., alkaline phosphatase) is stimulated while

Table 3. Optimal toxicity assay considerations

- 1. Verification components
 Ecosystem relevance
 Species sensitivity patterns
 Appropriate test phase
 Short or long exposure period
 Definitive response dynamics
- 2. Resource components
 Organism availability
 Laboratory availability
 Expertise required
 Expense and time required
- 3. Standardization components
 Approved standard methods
 Reference data base
 Interlaboratory validation
 Quality assurance and quality control criteria

another (e.g., galactosidase) is inhibited. When comparing test samples with reference samples, inhibitory *and* stimulatory effects should be regarded as a perturbation. Because microorganisms reproduce and adapt relatively quickly, pollutant effects must be greater than macrofaunal responses to be environmentally significant. In addition, as they respond readily to stimuli other than contaminants, caution should be used in data interpretations.

Mircobial assays can be divided into testing groups of either indigenous communities or laboratory-cultured strains and assay endpoints that are biochemical (such as enzyme activity, bioluminescence, lipopolysaccharides, muramic acid, and ATP content) or other metabolic processes (such as growth, uptake, respiration, substrate transformation, viability, and microcalorimetry). These endpoints have been measured by a multitude of methods, primarily in studies of water, wastewater, and soil systems [164,166–168,176,177,287,293–309] and have been applied to sediment systems to some degree [26,140,145,146,169,175,188,189,195, 274,277,310–317].

Pure culture systems used in assessments of sediment extracts include Microtox (*Photobacterium phosphoreum*) [274], *Spirillum volutans* [297], *Escherichia coli* [312], *Nitrobacter* sp. [318], *Azotobacter vinelandii* [319], *Aeromonas hydrophila* [298], *Pseudomonas fluorescens* [320], and *Pseudomonas putida* [177]. In most comparative surveys, but not in others, *Spirillum* was the least sensitive to toxicity [169]. Microtox has been listed by the EPA as a supplemental test to use in Tier 1 screening tests in the *Technical Support Document for Water Quality-Based Toxics Control* approach

[321]. However, limited use is actually made of any microbial toxicity test in EPA program activities [287,293].

Microtox testing has recently been incorporated into sediment toxicity test batteries [104,139,175, 178,308,322] and was originally reported in marine sediment studies by Schiewe et al. [274]. They compared extracting solutions and suggested dimethyl sulfoxide (DMSO) be used with caution. Response was related to the sums of organic class fractions contaminating the sediments. Some toxicity was attributed to extraction of natural organics [323]. The insensitivity to some elements and compounds might have been due to an inappropriate diluent (ionic strength adjustor) [104,308,324]. Interstitial water in large grain contaminated sediments was more toxic than fine-grain waters; however, the opposite was true for solvent-extracted sediments [308]. Numerous comparisons of Microtox sensitivity to pure compounds and effluents with Daphnia sp. and fish (primarily P. promelas) show similar effect levels in general [176,325] and were more sensitive than other microbial tests [326]. Many of these studies show Microtox to be slightly less (up to 1.5 orders of magnitude) sensitive, even though high correlation coefficients were observed [176]. In three [139,175,322] of the four [104] sediment comparison studies, Microtox responses were very sensitive and discriminatory of sediments contaminated with a wide variety of synthetic organic and metal compounds. The fourth study [104], in which the primary toxicant was ammonia, showed interstitial water effects to P. promelas, C. dubia, and S. capricornutum but not P. phosphoreum. Recently, a whole sediment exposure method using P. phosphoreum was presented and appeared to be more sensitive to hydrophobic chemicals than the elutriate Microtox assay [327].

A few metabolic processes or enzymes involved in key metabolic systems have been measured in contaminated sediments. Capone et al. [328] spiked estuarine sediments with Hg, Pb, Ni, Cd, Cu, Zn, Cr, Mo, Fe, and As and followed methanogenesis, sulfate reduction, CO₂ evolution, and microbial biomass. In general, there was initial inhibition followed by stimulated responses, which was attributed to metal complexation, biotransformation, and inhibition of competing microflora. Carbon dioxide and CH₄ production (organic matter decomposition), Hg methylation, sulfate and nitrate reduction were measured in sediment microcosms designed to measure perturbations [329]. Sediment trays incubated in situ in subarctic marine sedi-

ments were used to show that crude oil decreased nitrogen fixation, denitrification, and redox potential. Carbon dioxide and CH₄ production was increased [189]. In addition, some hydrolases were depressed while others were stimulated [188]. Similar effects occurred in river sediments impacted by crude oil with decreased phosphatase activity and elevated CO₂ and methane production [330], while others have seen no appreciable impact on oil-impacted microbial communities [310]. Water: sediment-phase marine microcosm tests were combined with in situ sample collection to study organic compound fate and effects [189]. Endpoints included microbe grouping, enzyme activities, ATP, and radiotracer analysis. Sayler et al. [314-316] conducted stream, microcosm, and experimental pond evaluations of sediment microbial community responses in perturbed systems. Accurate differentiation of contaminated and noncontaminated sediments required several community response endpoints (such as ATP, alkaline phosphatase, viable numbers, and mineralization rates).

Buikema et al. [26] compared glucose-G-phosphate dehydrogenase, catalase, acetylcholinesterase, ribonuclease, and acid phosphatase activities in extracted and unextracted lake sediments contaminated with metals and compared them to the survival of D. magna and H. limbata (48 and 96 h, respectively). Enzyme inactivation by solids was noted. Responses of catalase and H. limbata were related, and survival was inhibited in fine-grain sediments ($< 8 \mu m$), which may have been a particle size rather than a contaminant effect. Burton and Stemmer [146] summarized five stream profile assessments across the United States in which indigenous oxidoreductase and hydrolase activities in waters and sediments were compared to in situ chemical concentrations, biological community metrics, and surrogate test species. At each site, statistically significant relationships were observed between indigenous enzyme activities and in situ conditions, revealing toxicant impacts, natural spatial variation, and apparent food-web interaction. β-Galactosidase activity showed significant relationships in 80% of the studies to 37.5% of the measured stream parameters. β -Glucosidase and dehydrogenase activities were significant indicators of stream conditions in 60% of the studies, whereas reproduction (water only) of C. dubia was related in 50% of the studies to 22.5% of the stream parameters. Hydrolases were also effectively used to define sediment spatial variance in creosote-contaminated sediments [195]. It appears that, in general, assays of microbial processes and natural assemblages of microorganisms are superior to pure cultures as test systems [331].

Protozoa

Pratt and Cairns [332] grouped freshwater protozoa into six functional groups based on food requirements: dissolved mineral nutrients; bacteria and detritus; algae, bacteria, and detritus; diatoms; dissolved organics; and rotifers and protozoans. The "bacteria and detritus" group comprises the majority of protozoan genera. An assemblage of protozoa is a complex structure of herbivores, carnivores, omnivores, and detritus feeders [333]. The majority of species are cosmopolitan in nature and tolerate a wide range of ionic concentrations in fresh water [271]. They play an important role in the food web and "microbial loop" [149,271]. Holophytic and saprozoic species are producers using dissolved nutrients and are food for meiofauna as are holozoic species, which consume particulate living and dead material [271]. Few sediment contamination studies have been conducted with protozoans. Acute toxicity assays using the ciliate Tetrahymena sp. have involved only waters [334]. Assays of Colpidium campylum measured growth (14-25 h) in elutriates and sediment slurries [149, 335]. Protozoan colonization of artificial substrates (polyurethane foam) were used in laboratory and in situ tests [144,178,278]. Laboratory tests were with elutriates, whereas in situ assays were suspended over sediments. Community structure and functional endpoints included decolonization, protozoan abundance, taxa number, phototroph and heterotroph abundance, respiration, and island-epicenter colonization rates. Functional endpoints and phototrophs were the most sensitive endpoints. Stimulatory and inhibitory results were observed, and careful interpretation of effects was required [278]. Faster recovery occurred after diquat application in microcosms containing sediments [336].

Rotifers

Most rotifers are omnivorous, ingesting all organic particles of the appropriate size [333] and occur in an extreme variety of freshwater habitats. Few species exist, however, in fast-flowing streams. They are a food source for a few protozoans, large cladocerans, copepods, and small fish. Densities of 40 to 500 organisms per liter are common in littoral areas of ponds and lakes and are related to food

availability [333]. Recently *Brachionus calycifloras* has been used to measure toxicity of sediment elutriates [175], measuring survival at 24 h [337].

Nematodes

Little is known about free-living freshwater nematodes [271]. Many species are cosmopolitan in nature, can survive in a wide variety of conditions, and are primarily in the meiobenthos. They may reach densities of 100,000/m² to a depth of 2 cm in soft sediments. They can survive anoxic conditions for weeks and have highly resistant eggs [271]. Sediment extracts and elutriate toxicity were evaluated with Panagrellus redivivus in 4-d exposures [178,338-340]. Survival, growth inhibition, and molting inhibition (mutagenicity) were followed in tests begun with the second embryonic stage [338]. A free-living nematode, Caenorhabditis elegans, was recently proposed as a promising test system based on culture-test simplicity and sensitive 96-h LC50 values for several metals [341]. In situ community structure of nematodes was related to physicochemical factors such as nutrients and dissolved oxygen [342].

Bryozoans

Bryozoans are found on substrates in unpolluted, unsilted, well-oxygenated waters of lentic and slow-moving lotic systems. A colony may consist of thousands of individuals and is often associated with logs and stones in dim light [271]. Pardue and Wood [343] showed three species of phylactolaemate bryozoan ancestrulae were exposed for 96 h to Cu, Cd, Cr, and Zn and were more sensitive than several invertebrates and fish. Absence of a pollution-sensitive species, *Pectinatella magnifica*, has been correlated with poor habitat quality [344].

Gastropods

Most aquatic systems contain snails or univalve mollusks [271]. The species are selective for four different types of substrates: clean cobble, silt and detritus, macrophytes and associated detritus, and allochthonous organic matter [271]. Most are herbivorous, whereas *Lymanaea* is omnivorous. In water-only exposures, snails are very sensitive to copper and other metals [173]. Greatest toxicity was observed in flow-through, softwater systems using nonoperculate species; however, there were exceptions to each of these findings [173].

Pelecypods

Bivalve mollusks are common in large rivers and vary in size from 2 to 250 mm in length. Their primary food is fine organic detritus that has been resuspended [271], with the significance of plankton as a food varying with the species and ecosystem. Particles as small as 1 μ m can be removed from the water. Some species burrow during their life cycle well below the sediment surface (up to 25) cm) and have an interstitial water suspensionfeeding mechanism. Their life cycles range from one month to three years and are a common food of fish, reptiles, amphibians, and mammals. Their filtration capacity is massive. It has been estimated that approximately 7 billion clams inhabit Lake St. Clair, and they theoretically filter the entire lake every 13 d, assuming each organism filters 4 L/d [201]. This has dramatic implications on their role in ecosystem dynamics.

A drastic decline in species and population numbers of this ecologically and economically important group has been recorded in the past three decades [271]. Recently, they have been used in surveys of aquatic toxicity both in the laboratory and in situ [345]. Preference-avoidance tests with Acroneuria and insecticides showed increased drift and locomotor activity [346]. Sublethal alterations in oxygen consumption and free amino acid concentrations of Corbicula fluminea were studied in 60-d sediment-void systems [347]. While celluolytic activity was sensitive to effluent toxicity in laboratory and field caged experiments, longer exposures were necessary in the lab to elicit response levels noted in situ [348]. Mussels have been useful in long-term field-monitoring studies [219], and growth in situ appears to be a sensitive endpoint [349]. Keller and Zam [350] reported a simplified method for in vitro culturing of Anodonta, Lampsilis, and Villosa spp. Mussels have been used to a limited extent in environmental assessments [351]. Recently a toxicity test using young Anodonta imbecilis was reported and found useful for measuring sediment toxicity [352].

Oligochaetes

Oligochaetes act as do terrestrial earthworms, mixing surface layers of sediment. The tubificids are common in polluted areas [271]. They have been shown to uniformly mix surface layers [201,289] and play a major role in cycling of metals and organics out of the sediments [113,201,353]. Oligochaetes are a major component of benthic systems

in many aquatic systems [354] and transport deeper sediments to the surface as fecal pellets [114,115, 217]. The "aquatic earthworms" used for freshwater sediment toxicity assessments are limited primarily to *Tubifex* sp. *Tubifex tubifex* is considered as an indicator of organic pollution, particularly in waters with low dissolved oxygen saturation [271]. *Limnodrilus* sp. is tolerant of high metal concentrations [201]. *Lumbriculus* has been used in whole sediment tests to a limited extent [217,355], as have some other species in Sweden [356].

Oligochaetes represent a large portion of benthic biomass in some systems [356,357]. However, their usefulness as sediment toxicity indicators has received mixed reviews [156,356,357]. Their identification, variable species sensitivity, and fragility make them difficult to use [356,357]. Wiederholm et al. [356] followed growth and reproduction of five species for 0.5 to 1.5 years and found that contaminated oligotrophic sediments produced greater responses and reproduction was a more sensitive endpoint than growth. Tubifex tubifex survived for three months in sediments that were acutely toxic to D. magna; however, Tubifex growth and reproduction were inhibited and indicative of in situ infaunal community structure [156]. Use of Limnodrilus and Stylodrilus 96-h EC50 burrowing avoidance was a good indicator of contamination [358]. Tubifex tubifex and Limnodrilus hoffmeisteri avoidance behavior was observed in Cu- and Zn-spiked sediments [359]. The oligotrophic Stylodrilus heringianus displayed an ability to acclimate to sediment perturbations such as mixing, and based on reworking rates, mortalities, and dry weights showed sensitivity to mixed sediment contaminants [360] and endrin-spiked sediments [358]. Water-only exposures (10-14 d) of Acolosoma headleyi showed Cd had effects on growth. Acute toxicity sensitivity was similar to other conventional surrogates, but chronic effects were less sensitive [361].

Cladocerans

The importance of cladocerans in aquatic systems has been well documented since 1883 [271]. They play a significant role in the food web, phytoplankton and protozoan dynamics and fish stomach contents varying from 1 to 95% Cladocera by volume [271,362]. Some species are selective filter feeders, while others, for example, *D. magna*, are nonselective. An extensive database exists for pure compound toxicity testing with *Daphnia* sp. and its relative sensitivity compared to that of

other organisms [363]. Daphnia are well recognized as useful toxicity test species [147,219] due to their sensitivity to toxicants and ease of culture. In addition, standard methods exist for effluent and pure compound testing [364–366], while draft ASTM methods have been proposed for sediment assays [367]. These factors and the significant role Cladocerans have had in aquatic toxicology and criteria development make them obvious candidates for routine sediment toxicity assessments [147,219].

Daphnia magna and Ceriodaphnia are planktonic; however, in sediment assays they spend an extensive amount of time feeding on the sediment surface [219]. Daphnia magna, a nonselective filter feeder, ingests sediment particles [75] down to $0.5 \mu m$ [368], whether suspended or settled; thus, in sediment assays it functions as an epibenthic species. Relative sensitivity of D. magna to a wide variety of contaminants in whole sediment interstitial water, elutriate, and suspended sediment assays is well established [18,19,23,24,27,28,74,75,110, 124,139,140,145,147,156,195,205,206,245,247,250].

Fewer assays have been reported with C. dubia [140,175], which is commonly used in determining the chronic toxicity of effluents by using the threebrood (7-d) survival and reproduction test [364]. In most comparative studies, the acute and chronic toxicity sensitivity of C. dubia appears to be slightly greater than that of D. magna [144,175,369]. Some laboratories have reported occasional culture problems with C. dubia that can be traced to their water and food quality requirements [370]. A principal advantage of C. dubia over D. magna is its rapid reproductive rate after birth, allowing for sensitive measures of reproductive impairment within 7 d. In addition, smaller test volumes are needed for C. dubia. Reproductive effects may be studied in D. magna three-brood (7-d) assays by initiating the test with 5-d-old organisms [219,369,371].

Most sediment assays with the cladocerans have measured acute toxicity [124,219] in 48-h static (1:4 sediment-to-water ratio) systems. The original studies and some more recent ones used recirculating systems to study whole sediment, suspended sediment, or slurry effects [18,75,110]. In suspended sediment assays, some stress was observed due to recirculation and high turbidity [110]. The recirculating systems used lower sediment-to-water ratios, for example, 1:9.5 [18,219]. Recently subchronic toxicity studies of whole sediments and elutriates have been conducted with *D. magna* and *C. dubia*. These assays were static renewal (only over-

lying water was replaced in whole sediment assays) and measured survival, reproduction, growth, and pigmentation as endpoints [260,372] These com parative studies showed the cladocerans to be useful assays, sensitive and discriminatory of a variety of contaminated sediments [23,27,124,175,251] It is of interest that responses were often similar to those of benthic species both in laboratory assays and with in situ communities [139,144,175,245, 251], as well as in pure compound and ambient water studies [39,171,363] Many comparative studies have recommended D magna as an optimal screening species and as a routine sediment toxicity screening tool [9,27,147,175,219] Responses of D magna were observed in several pure compound studies to be good predictors of fish responses (r >88% [373]), poorly related to rainbow trout responses, and similar to responses of P promelas, or were compound-specific in their similarity with fish responses [171,374] Responses of D magna in sediment assays have also been correlated effectively to the concentration of contaminants in whole sediments, pore water, or those dissolved from the sediment to overlying waters [23,27,251]

Isopods

There are about 130 freshwater species of iso pods (aquatic sow or pill bugs), and little is known of American species [271] They are found primarily in shallows of small lakes, streams, and hyporheic, interstitial, and subterranean waters and usually are not of importance in the diet of fishes [271] The only reported sediment toxicity studies with isopods used field-collected *Asellus* in whole sediment recirculating systems [18,19,23,24] *Asellus* was usually not as sensitive as *D magna* or *H limbata* [18,19,23,24] A lack of culturing methods has limited their use in toxicity studies

Amphipods

Approximately 150 freshwater species of "scuds," or "sideswimmers," have been identified [271] The dominant species are *H azteca, Gammarus pseudolimnaeus, Gammarus fasciatus, Crangonyx gracillus*, and in the Great Lakes, *Pontoporeia hoyi* (now *Diporeia* sp.) Amphipods are widely distributed and common in unpolluted lotic and lentic systems, however, they are less common in large rivers *Hyalella azteca* is found in waters varying in hardness, pH, and salinity [271, 375] Amphipods are a primary food source for fish and voracious feeders of animal, plant, and detrital material [271] The epibenthic species, *H azteca*, has been used frequently in sediment tox-

icity testing [140,175,219,253,259,260,265,266,376] It has a minimum of nine instars, with the first five comprising the juvenile stage and a life cycle of less than a year [271] The amphipod juvenile stage of G pulex is more sensitive to sediment contamination than the adults [267,377] It is easily cultured [219,267,378] Standard methods were recently developed for whole sediment testing with H azteca [267] Hyalella azteca and Gammarus spp have been used frequently in acute toxicity studies of pure compounds or ambient waters [379,380] and found to be relatively sensitive in comparative studies [175,253,381] Pontoporeia sp has been used in Great Lakes studies because it is a primary benthic species there [15,16,27], unfortunately, it is not yet culturable, thus deep-water collections must be made for testing and tests conducted at approximately 4°C Extensive use has been made of P hoyi in bioaccumulation studies [96,143]

Sediment testing with H azteca has consisted primarily of whole sediment exposures (1 4 ratios of sediment to water) in static renewal systems for 7-, 10-, 14-, 28-, or 29-d periods [9,140,175,219, 259,267] Su₁vival is most frequently used as the endpoint in studies, however, in 29-d chronic exposure, growth and reproductive maturation are measured [253,259]

Pure chemical acute toxicity comparisons with other common test species, using Cd, NH₃, and phenol, showed Gammarus sp was less sensitive than the mayfly, Baetis, but more so than two midge (C riparius and Limnodrilus), the caddisfly, Hydropsyche, and the flatworm Polycelis [252,377, 381] A data review of 271 chemical toxicities to 57 species showed Gammarus was a poor predictor of crustacean or insect toxicity response [171] Many of these studies, however, were conducted on insensitive or unknown life stages, which may significantly affect sensitivity comparisons Large juveniles to young adults of H azteca and B lacustris adults were less sensitive than D magna or C tentans to Cu in sediment spiked sediment 10-d exposures [74] Hyalella azteca was more sensitive than D magna to Cd in static spiked sediment tests, and only free Cd contributed to toxicity Reduced toxicity was observed in flow-through tests [259,265] Hyalella azteca was one of the most sensitive and discriminatory of 20 different sediment toxicity assays in studies of three contaminated Great Lakes areas [175,253,259], and has been recommended as a tool to measure acute [219] and chronic toxicity [9,259,380] Useful chronic toxicity measures have consisted of H azteca growth (28 d) [259,267] and the Scope for Growth assay G

pulex which measures energy-absorbed vs. energy-metabolized (respired) [380,382]. Ingersoll and Nelson found survival and growth responses were similar in exposure periods ranging to 29 d. Survival decreased in some contaminated whole sediments with increased exposure time but not in others [259].

Insects

The mayfly (Ephemeroptera), H. limbata, and midges (Diptera) such as C. tentans and C. riparius have been used in sediment toxicity testing. The nymph stage of H. limbata – the life phase of interest – may last from one to two years [272] with numerous molts. Most mayfly nymphs are collectors or scrapers with possibly some filtering at the mouth of their burrow, and have a wide geographic distribution [272]. The nymphs dwell in tubes and are exposed in sediment and interstitial and overlying waters [383]. They prefer fine-particle-sized, organically enriched substrates; however, early instars have been observed in coarse-grain sediments [384]. The Diptera larval stage is primarily aquatic in all types of waters. Larvae go through four instars before pupation, each about one week in duration [267]. They burrow in the upper 10 cm of sediment, are omnivorous [267], and are an important food source for fish [271].

Hexagenia limbata has been used since the late 1970s in sediment toxicity evaluations [23,27,42, 122,124,219,245,251,385-387] and is sensitive to the presence of toxicants both in laboratory and in field surveys [245,251,385]. Most testing has used field-collected organisms as they are difficult to culture and may only reproduce once per year or two. Testing has been done in water, interstitial water, elutriate, artificial burrows, and whole sediment systems using static, static renewal, and recirculating for normally 10-d periods [251,386]. The measured endpoints include mortality, molting, and avoidance [23,27,175,245,385,387]. Hexagenia has been shown to be more sensitive than other simultaneously tested species (such as C. tentans, P. promelas, Asellus) [19,24,245], and their responses correlated with those of other species [245]. Their responses have also been representative of contaminant concentrations in the sediment extracts [24,245], whole sediments [19,28], and with in situ community profiles [245]. A failure of acute responses in the laboratory to correlate with in situ population distributions in contaminated areas was attributed to comparing acute 10-d exposures with possibly in situ chronic effects [251,385]. Some comparisons showed it less sensitive than D.

magna [124], but sensitivity was increased with increased exposure time (5-10 d) [251,385]. The burrowing behavior of *Hexagenia* alters Eh, pH, organic carbon, and contaminant profiles [122] and affects overlying water toxicant concentrations and toxicity to zooplankton [124]. The International Joint Commission (IJC) [9] recommends the use of *Hyalella* in sediment evaluations of 14-d exposures at 20°C.

Solid-phase testing with Chironomus sp. was first reported by Wentsel et al. [20,22]. Unlike H. limbata, midge can be as easily cultured [219,267, 378] as H. azteca, and there is also a standard sediment test guide available for C. tentans and C. riparius [267]. Chironomus sp. has been widely used in water, interstitial water, elutriate, and whole sediment assays ranging from 48-h to 29-d exposures [9,40,219,245,253,259,388-393]. Wentsel et al. measured growth (length) of C. tentans using early instars in 17-d tests and found responses were correlated with bulk metal concentrations [20,21]. Emergence of mature larvae was also related to metal contamination [22]. Ten-day exposures are optimal [219]. The larval stage is the most sensitive in chironomid life stage, and within that stage the first instar is the most sensitive for C. tentans and C. riparius, although the second instar is often used [219,259,267,388,389]. The most common endpoints include mortality and growth (dry weight) [219,245]. The IJC recommends [9] growth and emergence of C. tentans beginning with a 13d-old organism and continuing for 10 d or emergence. Nebeker et al. recommend beginning with 10-d-old organisms and continuing the assay for 15 d [219].

Chironomid sp. may reside in relatively polluted areas and, as would be expected, are often more resistant to toxicants than many other test species [74]. Chironomus riparius is common in the Great Lakes [272,394]. Sublethal response (growth) was correlated with Microtox effect concentrations, H. limbata or D. magna response, benthic community health, and also discriminated areas of contamination [139,245]. The primary route of uptake and resulting toxicity of Kepone was via the interstitial water and was controlled by organic carbon partitioning [40]. Recent comparative testing found C. riparius was more sensitive than C. tentans in several contaminated whole sediment assays [175,266]. Other tested species include Chironomus decorus [390] and Paratanytarsus parthenogeneticus [395, 396]. Chironomus sp. has been recommended as a routine whole-sediment [219] and interstitial-water [147] toxicity test species.

Fish

Toxicity testing with fish in sediment systems has been limited primarily to the fathead minnow (P. promelas); however, other species have been used, such as the rainbow trout (S. gairdneri/O. mykiss), goldfish (Carassius auratus), largemouth bass (Micropterus salmoides), and bluegill (Lepomis macrochirus) [27,32,397]. P. promelas has a widespread geographic distribution, it is easily cultured and has been widely used in the development and validation of water quality criteria [363], pure compound, and effluent testing [398], standard methods exist for water exposures [364-366]; it is an obvious choice for routine sediment testing. A significant amount of fish testing has focused on bioaccumulation of sediment-associated toxicants. These investigations have involved laboratory and in situ exposures usually ranging from 10 to 28 d [399]. Method guides have been published for 10d sediment bioaccumulation tests of P. promelas [400]. In addition, fish are the principal focus of biomarker studies using a wide range of genotoxicity, biochemical, and histopathological endpoints indicative of sublethal exposures to sediment contaminants [3].

Most sediment testing has been acute exposures (96 h) to the adult, which is relatively insensitive [23,250] as compared to early life-stage and full life-cycle endpoints [255,256]. Norberg-King and Mount [256] developed a 7-d subchronic larval survival and growth assay for effluent testing that has been adapted for sediment extracts [165], elutriate [268], interstitial water [104], and whole sediment assays [141,175,261,264,401]. Another 7-d early life stage assay developed by Birge et al. [17] begins with the embryo stage and continues through 3 d of larval development with endpoints of survival, teratogenicity, and growth (length). This assay was used in whole sediment testing [264] and, as the larval growth assay, has been found to be a useful and sensitive sediment assay [165,175].

Embryos and larvae are exposed to overlying water, interstitial water, and ingested sediment [32]. Because the embryo stage is susceptible to fungal infections, it is necessary to aerate overlying waters gently [264]. As in the cladoceran whole sediment assays, larvae tend to feed extensively on the sediment surface during the test, thereby potentially increasing their exposure to sediment-related contaminants. Increased uptake of PCB is attributed to fish's "mouthing" sediments and desorption occurring in their buccal cavity [402]. Sediments are a spawning substrate for many pelagic and epi-

benthic organisms, so that effects such as reproductive behavior, hatchability, development (terata), and growth are critical endpoints to monitor.

Correlations between endpoints and sediment contaminant levels have been reported in some studies [32,264] and not in others [23,24,264]. Pure chemical toxicity data evaluations comparing *P. promelas*, rainbow trout, and bluegill showed fish surrogates were good predictors of fish response [398] with increasing similarity being related to the degree of taxonomic similarity [171]. Endpoints in Zn-contaminated sediment exposures ranked as teratogenicity, growth, and mortality in order of sensitivity [165]. Terata EC50s in *P. promelas* were four to six times lower than frog embryo terata effect levels [165]. The cough response in interstitial water exposures to bluegills was difficult to interpret [27].

Amphibians

A limited number of sediment toxicity studies of amphibians have been reported [264,397]. Dawson et al. [165] used the frog embryo teratogenesis assay (FETAX) with Xenopus laevis to measure sediment extract effects from Zn-contaminated sediments. Sediments were extracted for 24 h in reconstituted water at various pH levels. EC50 levels for terata were from 2.5 to 3.6 mg/L Zn at 100 mg/L hardness, 2.0 to 4.2 mg/L for growth, and 34.5 mg/L for survival. Peddicord and McFarland [397] exposed Bufo boreas to suspended sediments (2-20 g/L) for 21 d. Whole sediment embryo studies were conducted on the leopard frog and narrow-mouthed toad (Gastrophoryne carolinesis) [32,264]. Tissue concentrations were related to metal exposure but not mortality. The duration of embryo contact with the sediment appeared to be an important factor mediating exposure.

Algae

In lotic and some lentic systems, phytoplankton are the major primary productivity source [148,403]. Benthic-associated algae (periphyton) dominate primary production in many streams [191] and shallow lake regions [99]. The critical role of algae in ecosystem functioning has been demonstrated for many years through limiting-nutrient, eutrophication, and primary productivity studies [362,403]. Algae are also a basic fisheries resource via zooplankton grazing [403,404], provide a major carbon source for the sediment microbial food web [129], and cycle nutrients and toxicants [362,405]. As with microbial metabolic endpoints, photo-

synthetic organisms frequently show stimulatory activity in response to nutrients or perhaps alterations of feedback mechanisms [292]. Stimulatory responses are as useful an endpoint in the assessment process as is inhibition.

Algae have been proposed as surrogates for plants [147]; however, this generalization is unrealistic as shown with herbicide compounds [406]. An evaluation of the sensitivity of 16 microalgal strains to 19 compounds revealed that no one species had commonly observed sensitivity patterns and recommended testing a wide range of taxonomic types of algae [407]. Interlaboratory comparisons of assay responses are difficult due to differing methodologies [408]. A review of the freshwater algal literature found results vary by three orders of magnitude due to physical and chemical methodological parameters. Biomass and gas exchange differences due to shaking or continuous aeration effects on CO₂ limitation and pH were the apparent cause of this variation [408]. This sensitivity to the test environment reflects the rapid uptake and metabolism shown to a greater extent in microbial assays. As with microbial systems, stimulatory responses may occur and responses also change with incubation time responses [175]. Temporal effects are likely due to nutrient limitation, changing water quality, toxicant availability, and adaptation [408,409].

As with the protozoan and rotifer assays, most sediment quality testing with planktonic algae have used elutriates, interstitial water, or overlying waters [147,148,410]. The principal test species has been *S. capricornutum* and consisted of the standard growth assay for 96 h [364]. Other assays with this species have used 48-h incubation periods [175] or measured photosynthesis based on ¹⁴CO₂ assimilation during a 24-h period [178,410]. Test battery studies of pure chemicals, effluents, and contaminated soils have shown *S. capricornutum* to be a sensitive test species [147,411–413].

Other phytoplankton assays that have been exposed to sediment elutriates or overlying waters include: (a) the algal fractionation bioassay (short [4 h] and long term [24–96 h]) with natural assemblages, in laboratory or in situ exposures, or precultures of micro- and ultraplankton, where ¹⁴CO₂ uptake and chlorophyll are measured [15,142,148, 410,414]; (b) microcomputer-based video analysis of chlorophyll fluorescence (4-h incubation) [142, 148]; (c) microplate ATP analyses [415]; and (d) flow cytometry measures of cell size or biochemical integrity [416]. In situ experimental pond studies showed filamentous algae were the most sensitive

species in pentachlorophenol-dosed systems [417]. Ponds dosed with trichloroethylene showed decreased phytoplankton diversity and increased abundance [418].

Also reported was whole sediment exposure to *Chlorella vulgaris* whereby sediments were carefully added to the bottom of the test vessel via tubing, and ¹⁴C assimilation was measured [279].

Attached algal (periphyton) communities are useful indicators of aquatic pollution [157,419-421], but are infrequently included in studies. See Steinman and McIntire [422] for a review of periphyton community responses and interactions in disturbed aquatic systems. Shifts in community structure from pollution-sensitive groups to tolerant groups occurred in streams receiving metal [157,420,421] and organic pollution [420] at low instream concentrations and showed a response gradient that was related to contaminant concentrations [157,421]. It was evident that EPA water quality criteria were not protective of 95% of aquatic life as designed [421]. Community structure changed from diatoms to green algae, which are of lower assimilatory efficiency, thus possibly affecting the higher level consumers [421]. A continuous flow in situ periphyton bioassay was described that measured nutrient limitation by using chlorophyll and ¹⁴CO₂ uptake [423]. Outdoor experimental stream periphyton communities were sensitive to ppb levels of pentachlorophenol, based on biomass and pigment production [424].

Macrophytes

Duckweed, *Lemna* sp., a nonrooted, floating, vascular aquatic macrophyte, has recently been used with sediment elutriates and whole sediment assays [175]. *Lemna minor* and *Lemna* sp. have a wide geographic distribution and are common in many lentic environments. *L. minor* has recently been proposed as an effluent test species where frond number and chlorophyll production were the most sensitive endpoints when compared to the responses of *C. dubia* and *P. promelas*, for some effluents [425]. Laboratory responses of *D. magna* and *L. minor* were correlated with pond mesocosm responses [426]. Other recommended endpoints have included root length and ¹⁴C uptake [425].

Klaine et al. [427] recently used a rooted aquatic macrophyte, *Hydrilla verticillata*, to measure toxicity in whole sediment assays with stream and lake sediments contaminated with a variety of metals and synthetic organics. Endpoints included root and shoot length, peroxidase, dehydrogenase, and chlorophyll. Some sediments showed plant growth

(root and shoots) to be sensitive to contamination, while others showed peroxidase activity to be most sensitive. As with all other photosynthetic system studies, both inhibitory and stimulatory effects were observed.

FIELD VALIDATION

As discussed earlier, laboratory manipulations (e.g., sediment mixing), extractions (e.g., elutriate, interstitial water), spiking and exposure conditions (e.g., static vs. renewal vs. flow through, natural vs. artificial light, whole sediment vs. interstitial vs. elutriate phase) will often affect toxicity responses [207,245,428]. In order to meet most study objectives, it is therefore necessary to validate laboratory responses with some degree of in situ chemical, physical, and biological monitoring [47,81,144,181, 219]. There are a variety of approaches to address this validation component of sediment quality assessments [9,35,39,141,181,429]. Validation may consist of comparisons to historical chemical and nekton or benthic community survey data, qualitative or quantitative biological surveys [9,12,39, 47,144], or in situ toxicity testing [81,238]. At this point in time, the science necessitates use of some degree of field validation of biological effects in which natural factors such as habitat, life cycles. and spatial and temporal variability are considered. The importance of this requirement, however, is not equal at all sites [35]. In severely contaminated areas, the relative degree of acute toxicity is of little matter, and in situ biological impacts are apparent, therefore, validation can be cursory in nature. However, at each severely contaminated site and at many other lesser contaminated sites are gray zones where acute toxicity decreases to sublethal to chronic effects. Field validations integrate water column and sediment effects, whereas laboratory sediment toxicity assays often isolate and measure only sediment toxicity effects. Realistic and accurate assessments of ecosystem health and sediment contamination in these areas will have to include in situ biological validation.

Community structure

Benthic communities have been the most widely used indicators of aquatic health [39,43,44] and, more recently, sediment quality indicators [9,430]. These communities are ideal indicators of water-sediment quality because they are relatively sedentary, are comprised of species ranging from pollution-sensitive to -tolerant, and occupy multiple trophic levels and a myriad of niches involved in ecosystem functioning [182]. Their life cycles

(exposure periods) range from hours to years, and an extensive data base exists to aid study design and data interpretation [43,430-432]. Because species presence/absence and composition are functions of numerous environmental factors such as varying organism life cycles, storm events, and habitat requirements, care must be used when attributing effects to pollution [43,52,183]. Many excellent reviews and references exist that describe effective approaches for benthic community monitoring [43,201,420,430-435] useful in sediment toxicity validation.

Other important communities such as microbes, meiofauna, phytoplankton, zooplankton, and fish have been used to a lesser degree in evaluations [4,43,44,142,148,149,359] involving sites that had contaminated sediments, and links to the sediments were more indirect than those found with benthic communities. They are useful in studies dealing with food chain transfer of pollutants, such as bioaccumulation processes.

In situ assays

A newer approach in studies of sediment contamination or validation of laboratory results is in situ assays. These may involve enclosures such as lake limnocorrals or in situ mesocosms [436–440] that partition a column of water to the sediment surface; artificially constructed streams allowed to colonize with biota indigenous to a nearby stream [281,438,441-443]; experimental ponds that are seeded and allowed to colonize with indigenous species [411,438,440,444,445]; placement of caged species in situ (plankton, mussels, zooplankton, leeches, fish) [81,148,238,281,289,401,438,439, 446-448]; placement of litter bags or leaf packs in situ [449-451]; or colonization of artificial substrates by periphyton, protozoa, or macroinvertebrates [144,175,278,419,451-455].

Of those approaches, few have focused directly on sediment toxicity effects [238]. The limnocorrals and experimental ponds have primarily been used to study environmental fate of pure compounds such as pesticides and polychlorinated dibenzo-compounds and have effectively demonstrated the influence of sediment partitioning and food chain effects [281,437,456]. As would be expected in test systems more closely mimicking the real world, variability is typically greater through time and between replicates than that observed in more controlled laboratory environments. Carbofurantreated ponds containing chambers of indigenous benthic invertebrates showed acute toxicity at 5 μ g/L [439]. In general, the trichopteran *Limnephi*-

lus was most sensitive, followed by H. azteca. Gammarus lacustris and C. tentans were more resistant than H. azteca, and the damselfy, Enallagma, was the least sensitive; however, all species were adversely affected [439].

Artificial substrates (including leaf packs and litter bags) allow one to study multiple trophic levels from a community structure and function perspective with a range of endpoints considered simple to complex. The uniform test system reduces habitat (substrate) effects, historical/temporal effects, and sample collection-laboratory related error. If one is interested primarily in sediment toxicity, however, the substrates create an artificial barrier and, thus, likely underestimate sediment effects on species that may contact the sediment during their life cycle.

An effective way to study single-species effects of contaminated sediments is possible with in situ sediment test chambers [238]. This approach was originally proposed by Nebeker et al. [219]; however, no studies were published on the in situ chamber approach until 1991 [238]. The approach is relatively simple; it removes sampling- and laboratory-induced error from the assessment process while maintaining in situ conditions whose importance in determining sediment toxicity might not be known, such as sunlight, diurnal effects of temperature and oxygen, sediment integrity, spatial and temporal variability effects, flow-through conditions with site water, resident meio-microfaunal interactions, and turbidity. Significant differences were observed between in situ and laboratory responses, with greater sediment toxicity and less overlying water toxicity occurring in the lab [238]. Site toxicity changed seasonally. Elutriate toxicity was generally less than that of whole sediment or interstitial waters, and filtration reduced toxicity significantly. Recent in situ studies with larvae of P. promelas [401,448] measured larval weight change in 7-d exposures. The larvae were more susceptible than C. dubia to turbidity-storm events but effectively demonstrated sediment toxicity and laboratory differences. Limitations to this approach include possible cage effects and food limitation in long-term exposures; deployment is difficult in deep or fast-moving waters.

CURRENT APPLICATIONS

The optimal design and use of sediment toxicity tests is dependent on the study objective(s). For example, use of the standard elutriate [10] as a test medium may be appropriate in toxicity screening of dredge materials. However, it is inappropriate if

considering bedded sediment toxicity due to resuspension effects, where the frequency of occurrence, embeddedness, suspended solid:water concentration, and exposure duration effects are important determinants of toxicity and quite different from an elutriate exposure. Other study objectives for which sediment toxicity assessments are appropriate and have been used include defining the spatial extent (both horizontal and vertical) of contamination, determining sensitive target species and communities, predicting or verifying chemical or dredge material effects, and serving as components of biomonitoring programs.

The state of the art is adequate to fulfill current study objectives of defining areas of acute toxicity in severely contaminated sites so that remediation or dredge material disposal options can be determined. These studies [140,141,147,175] and other site characterization assessments [139,268,457,458] have shown the utility of using sediment toxicity assays.

The EPA is considering toxicity testing of sediments as a component of several statutory programs to assist in managing contaminated sediments [136]. Recently, the EPA developed a sediment management strategy that affects multiple program activities such as criteria, effluent guidelines, point sources (including combined sewer overflows and storm waters), nonpoint sources, pesticide review, premanufacture chemical testing, PCB cleanups, corrective action at solid waste facilities, hazardous waste landfill remediation, natural resource damages, ocean disposal, and dredging activities [136]. It may be used in the National Pollutant Discharge Elimination System (NPDES) by incorporating it into permit requirements for municipal and industrial effluent discharges [136].

Toxicity identification evaluations (TIEs) are used by the NPDES program to identify the constituent(s) of the effluent that contribute to its acute toxicity [39]. These procedures are being modified for use with contaminated sediments and use interstitial water as the test phase [104]. Ammonia has been identified as a particularly toxic component that may frequently be identified in a TIE as the primary toxicant [104]. Toxicity assays and/or TIEs can be used in the assessment process of hazardous waste site remediations at Superfund sites, Resource Conservation and Recovery Act-(RCRA-)permitted facilities, or Great Lakes "Areas of Concern" [136].

Sediment testing has been widely used in evaluations of dredge material and mapping of channel sediment toxicity [458]. Pesticide registration

and reregistration require environmental fate and effect studies, which indirectly have addressed sediment toxicity, as have premanufacture reviews of new chemicals [459]. The EPA Criteria and Standards Division is developing sediment quality criteria using the equilibrium partitioning (EqP) approach. Sediment toxicity testing will be necessary to verify the EqP assumptions that interstitial waters are the primary route for benthic biota; toxic (bioavailable) fractions can be predicted with normalization factors such as TOC or AVSs; and water quality criteria are appropriate for interstitial water and whole sediments, being protective of benthic and nonbenthic species, community structure, and ecosystem functioning.

Sediment toxicity testing is also a component of other integrative approaches where in situ chemical contamination and biological communities are measured, in addition to toxicity testing. This approach may be used to produce sediment criteria or simply to assess the problem. Integrative approaches vary and include the apparent effects threshold and sediment quality triad [39], both of which were demonstrated effectively in marine systems [459,460]. The integrative approach is superior for accurate studies of ecosystem perturbations because each component assists interpretations and validation of the other component effects and, hence, better describes real-world conditions [47].

CONCLUSIONS

The optimal sediment toxicity assay is a relative measure that will vary between studies and change with development of new methods. The optimal assays in 1977 appeared to be those used by Prater and Anderson [19], demonstrating acute toxicity of whole sediments to H. limbata and D. magna, and the chronic assay with C. tentans of Wentsel et al. [20–22]. In 1991, the decision on an optimal design is complicated by advances in the science showing that numerous sensitive species and communities can be assayed for a variety of time periods in possibly four phases (whole sediment, interstitial water, elutriate, or extractable), under different exposure conditions (lab vs. in situ, static vs. flow through, mixed vs. unmixed sediment, and other varying physicochemical conditions), and monitoring several endpoints of lethal and sublethal toxicity. Undoubtedly, the future optimal assays will be superior to those available now. However, the criteria that influence the decision of which assays are optimal – such as What are the study objectives? What are the resource requirements? What type and degree of contamination is to be assessed? Which assays are standardized, relevant, sensitive, and discriminatory? — will change little (Table 3).

Other factors important in determining the optimal assay have been discussed [284,461] and include culturing requirements, ecological relevance, response range, discriminatory ability, replicability, and degree of standardization. A standard assay should have an adequate data base to provide quality assurance and control limits and thus decrease the artificial influence of the laboratory on the sample's toxicity. There is no one optimal assay for all assessments of sediment toxicity [462]. There are, however, optimal assays for particular situations. Acute toxicity testing is adequate for some situations and can predict ecosystem effects in cases where contaminants are relatively concentrated [175,179]; however, subchronic, chronic, and in situ testing will likely be required in areas of lesser contamination to adequately ascertain whether the ecosystem is being disturbed. Unfortunately, the optimal assay(s) can be proven only a posteriori, not a priori.

Every test site is a unique ecosystem, and the toxicity of the sediment will be a function of both independent and integrative natural factors (such as patch dynamics) and their biological, physical, and chemical relationships with the contaminants (as well as unknown contaminant interactions). It is essential, therefore, to use multiple assays in the assessment process, and a tiered integrative testing approach seems logical [48,152,284,463]. The use of multiple assays improves the chance of detecting toxicity; however, detection of, or lack of, toxicity does not ensure a valid assessment. "No amount of testing will eliminate all variability, and some probability of toxicity will have to be accepted" [171]. The detection of toxicity in the laboratory must be validated by removing the possible effect of collection-laboratory manipulation and then relating the effect to an ecosystem perturbation. It is well recognized that the sensitivity level of an assay is dependent on the toxicant mode of action, organism toxicokinetics, life stage, and measured endpoint. However, of equal importance is determining the appropriate level of sensitivity; that is, a level that is ecologically relevant (Fig. 2). This task is difficult, if not impossible at this time, to determine with confidence and will likely always be a point of debate. Nevertheless, it should be an objective of every sediment toxicity assessment.

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